TECHNICAL NOTE

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Resolution of Some Enantiomeric Amines of Forensic Interest by High-Performance Liquid Chromatography

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ABSTRACT: The high-performance liquid chromatographic resolution of the enantiomers of amphetamine, methamphetamine, ephedrine, and pseudoephedrine is described. The sugar isothiocyanate, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), is used as a chiral derivatizing agent, with chromatographic separations of the diastereomers formed with each amine made using a standard achiral C_{18} stationary phase.

KEYWORDS: toxicology, amphetamines, chromatographic analysis, chiral separation

The enantiomeric primary and secondary amines (amphetamine, methamphetamine, ephedrine, and pseudoephedrine) are an important class of drugs to the forensic drug chemist or toxicologist. Drug enantiomers often possess different pharmacological activities and different rates of disposition [*1,2*]. These properties are the result of chiral recognition sites on drug receptors and other biomolecules. In addition, one enantiomer may be under regulatory control while the other is not. The enantiomeric form of a drug sample may provide information concerning its origin or route of synthesis [*3*]. It has been reported that certain drugs derived from a natural source usually exist as one enantiomeric form, and clandestinely produced drugs are usually a mixture of enantiomers [*4*].

Various procedures have been developed for the determination of drug enantiomers [*5–8*]. Gas chromatography has proven to be a useful technique for resolution of amine enantiomers, either directly on optically active stationary phases or as diastereomers on achiral phases [*5,6*]. Liquid chromatographic procedures have also proven effective in the resolution of drug enantiomers using both normal phase and reversed phase techniques [*7,8*].

One method used in high-performance liquid chromatography (HPLC) to resolve enantiomers involves the conversion of the enantiomers to diastereomers using chiral derivatizing agents. The diastereomers may then be separated due to differences in their physical and chemical properties. A good chiral derivatizing agent may also serve to enhance detectability of the drug as well as improve the chromatography. The sugar isothiocyanate, 2,3,4,6-tetra-

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O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), has proven to be such a chiral derivatizing agent [9]. The GITC derivatizing method is simple; the chiral reagent is commercially available and chemically and stereochemically stable; and the thiourea derivatives have a high ultraviolet absorption.

This article describes the liquid chromatographic resolution of the enantiomers of amphetamine, methamphetamine, ephedrine, and pseudoephedrine. The enantiomeric amines were derivatized with GITC and the corresponding diastereomers separated using a standard achiral C_{18} stationary phase.

Experimental Procedure

Reagents and Chemicals

Samples of *d*-methamphetamine hydrochloride, *dl*-methamphetamine hydrochloride, *d*-amphetamine sulfate, *dl*-amphetamine sulfate, *l*-amphetamine, *d*- and *l*-pseudoephedrine, and *d*- and *l*-ephedrine were obtained from Sigma Chemical Co., St. Louis, MO. 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was purchased from Polysciences, Warrington, PA. HPLC-grade chloroform and methanol were obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. HPLC-grade tetrahydrofuran (THF) was obtained from Fisher Scientific Co., Fair Lawn, NJ. Water was double distilled. Other samples analyzed in this study were submitted to the Alabama Department of Forensic Sciences by various Alabama law enforcement agencies.

Instrumentation

The liquid chromatograph was a modular system and consisted of a Waters Associates (Milford, MA) Model 6000 A pump, Model U6K injector, Model 440 UV detector operated at 254 nm and 0.2 absorbance units full scale (AUFS), and Houston Instrument (Austin, TX) OmniScribe dual pen recorder. Infrared spectra were recorded on a Perkin Elmer (Norwalk, CT) Model 1500 Fourier transform infrared spectrophotometer (FT-IR).

Chromatographic Procedures

Reversed phase separations were carried out on a 15-cm by 4.6-mm inside diameter (ID) Econosphere C_{18}, 5-μm, column (Alltech-Applied Science, State College, PA) at ambient temperature. The analytical column was preceded by a 7-cm by 2.1-mm ID guard column dry-packed with Co:Pell ODS (Whatman Inc., Clifton, NJ). The mobile phases consisted of various mixtures of THF and water, or methanol and water. The mobile phase flow rate was 1.5 mL/min.

Derivatization Procedure

To a separatory funnel is added 1 mg of each amine enantiomer to be studied, or 2 mg of each racemic mixture. The amine is dissolved in 0.45*N* sodium hydroxide and extracted as the base into chloroform (2 by 25 mL). To the chloroform is added a 10% molar excess GITC in chloroform. The reaction is allowed to proceed at room temperature for 10 min. The reaction mixture is evaporated to dryness under a stream of air. The resulting residue is dissolved in 1 mL of THF or methanol and 5 μL injected into the liquid chromatograph.

Results and Discussion

Several reported HPLC methods illustrate the utility of GITC derivatization for the separation of the enantiomers of amphetamine, ephedrine, pseudoephedrine, and norephedrine

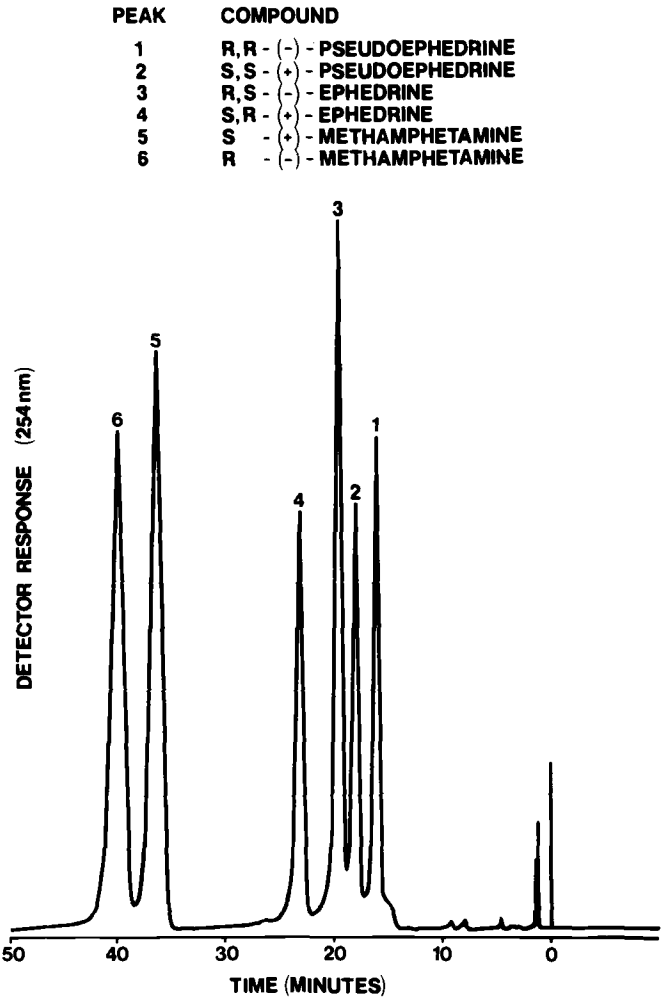


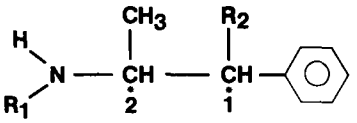
FIG. 1—*HPLC separation of GITC-derivatized amines using THF: water (3: 7) mobile phase. Peaks: (1) R,R-(−)-pseudoephedrine; (2) S,S-(+)-pseudoephedrine; (3) R,S-(−)-ephedrine; (4) S,R-(+)-ephedrine; (5) S-(+)-methamphetamine; (6) R-(−)-methamphetamine.*

[*10,11*]. However, these separations were achieved using different chromatographic conditions. A single procedure has not been described for the separation of the enantiomers of these drugs, several of which are encountered in combination in forensic sciences laboratories. The development of a single chromatographic method for separating a maximum number of these amine-GITC derivatives for routine determination of enantiomeric composition in forensic sciences laboratories was one of the initial goals of our study. The second major goal was the separation of methamphetamine-GITC derivatives.

Methamphetamine is one of the most frequently encountered drugs prepared in clandestine laboratories, and it is usually the racemic mixture (R,S-(±)-methamphetamine) because the typical synthetic methods involve formation of the asymmetric center in the molecule from two achiral starting materials. A second readily available source of methamphetamine is by extraction from Vicks® inhalers. This over-the-counter medication contains R-(−)-methamphetamine or *l*-desoxyephedrine. Methamphetamine produced by the pharmaceutical industry for prescription drugs (such as Desoxyn®) is the S-(+)-isomer. Thus, the determination of the enantiomeric composition of a methamphetamine sample can produce considerable information concerning its origin.

The GITC derivatization of the enantiomers of methamphetamine proceed as described for other secondary amines. The liquid chromatographic separation of the methamphetamine-GITC, ephedrine-GITC, and pseudoephedrine-GITC derivatives on a column packed with C_{18} chemically bonded silica using a mobile phase of THF:water (3:7) is shown in Fig. 1.

The elution order for methamphetamine is the S-(+)-isomer eluting first, followed by the R-(−)-isomer. The ephedrine molecules contain two chiral centers resulting in four possible stereoisomers, two sets of enantiomers. The erythro-isomers (R,S and S,R) are known as ephedrine, and the threo-isomers (R,R and S,S) are referred to as pseudoephedrine. The relationship between the erythro and threo forms is diastereomeric, one chiral center of the same configuration and one of opposite configuration. The diastereomers derived from GITC derivatization show the R,S-(−)-isomer of ephedrine to elute before the S,R-(+)-isomer. The elution order of pseudoephedrine was found to be the R,R-(−)-isomer eluting before the S,S-(+)-isomer. Amphetamine-GITC derivatives eluted after approximately 60



R_1	R_2	COMPOUND	ABSOLUTE CONFIGURATION
H	H	AMPHETAMINE	2S - (+) 2R - (-)
CH_3	H	METHAMPHETAMINE	2S - (+) 2R - (-)
CH_3	OH	EPHEDRINE	1R, 2S - (-) 1S, 2R - (+)
CH_3	OH	PSEUDOEPHEDRINE	1R, 2R - (-) 1S, 2S - (+)

FIG. 2—*Chemical structures, absolute configurations, and optical rotations of amines examined.*

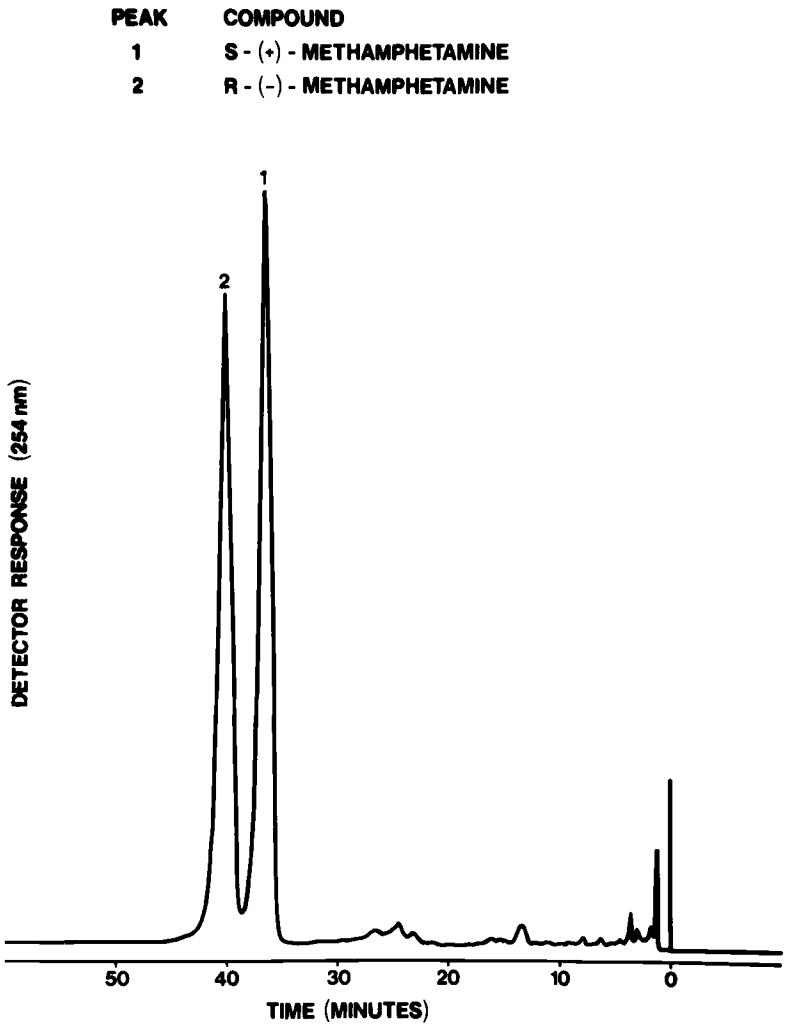


FIG. 3—*HPLC separation of GITC-derivatized racemic methamphetamine using THF: water (3: 7) mobile phase. Peaks: (1) S-(+)-methamphetamine; (2) R-(−)-methamphetamine.*

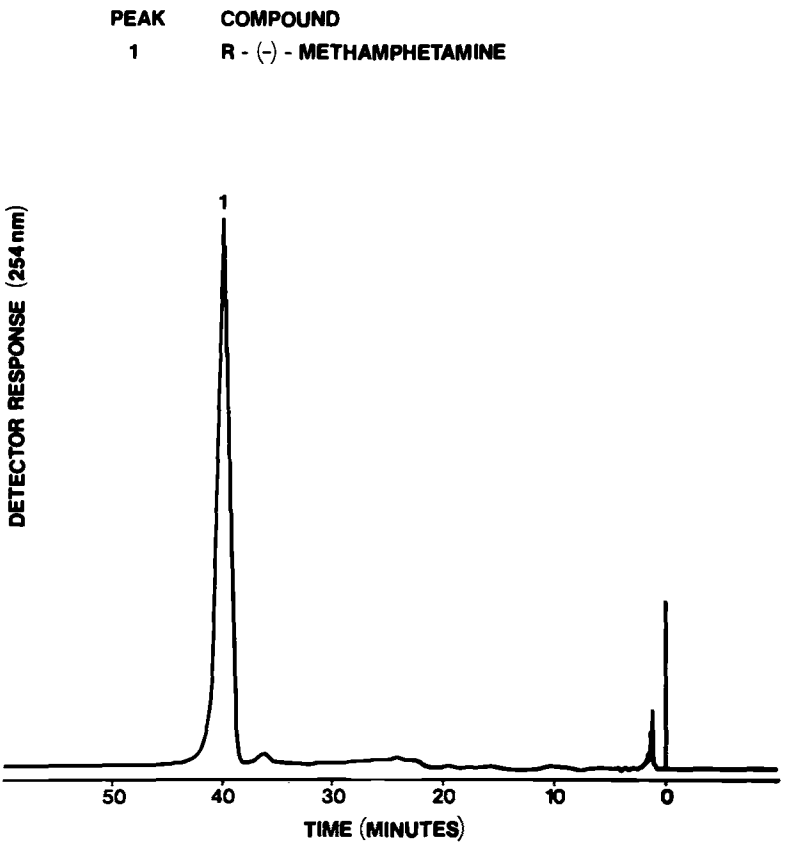


FIG. 4—*HPLC separation of GITC-derivatized sample submitted by law enforcement agency using THF: water (3: 7) mobile phase. Peak: (1) R-(−)-methamphetamine.*

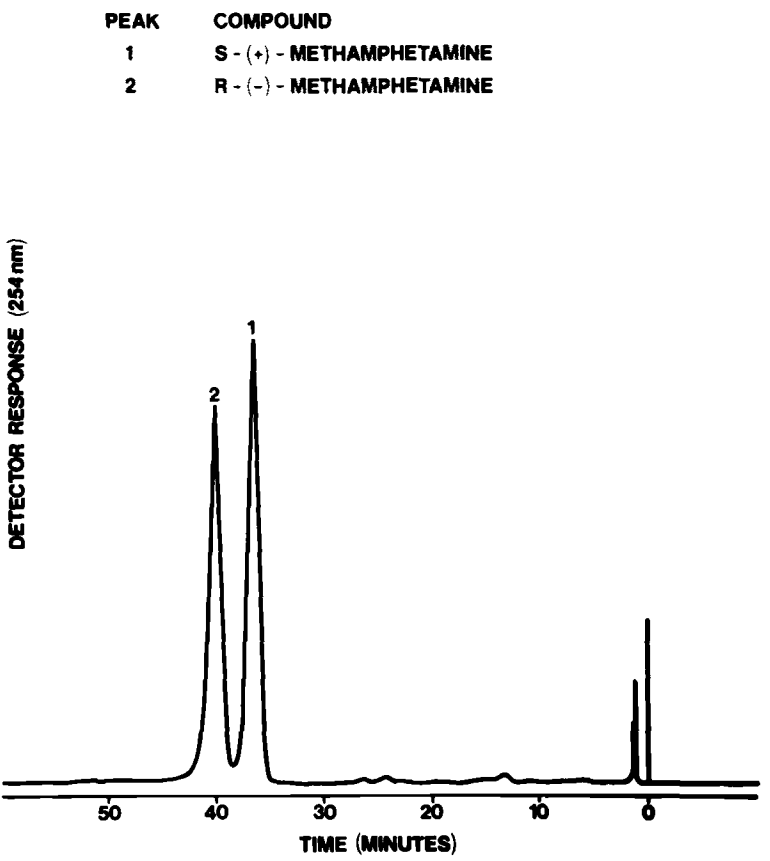


FIG. 5—*HPLC separation of GITC-derivatized sample from syringe found at clandestine methamphetamine laboratory using THF: water (3: 7) mobile phase. Peaks: (1) S-(+)-methamphetamine; (2) R-(−)-methamphetamine.*

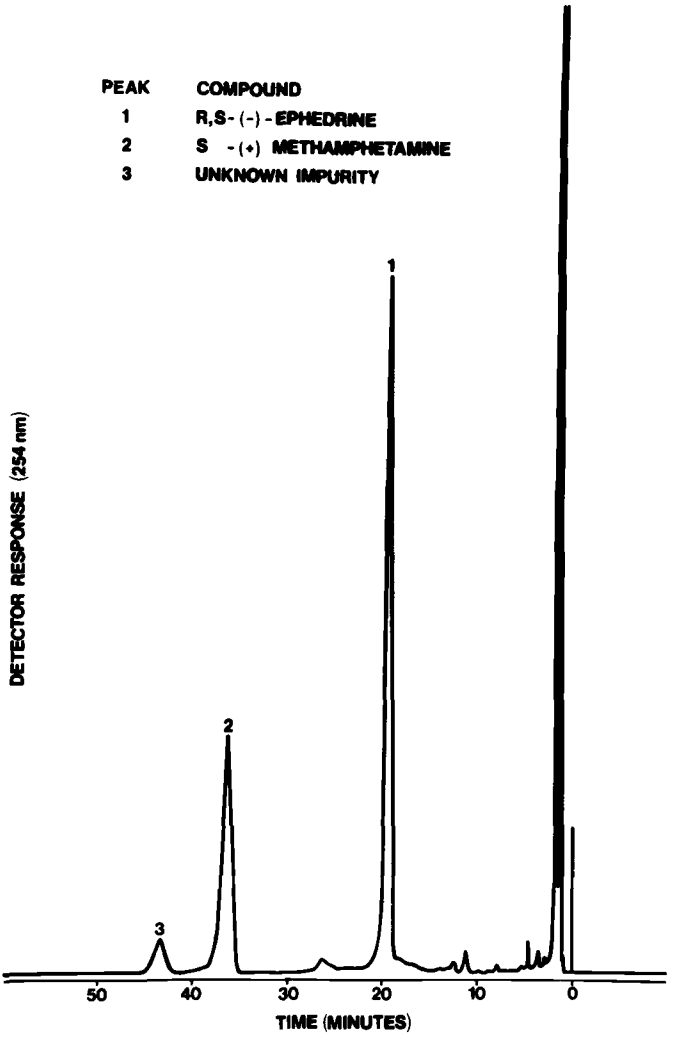


FIG. 6—*HPLC separation of GITC-derivatized sample purchased by undercover police officer using THF: water (3: 7). Peaks: (1) R,S-(−)-ephedrine; (2) S-(+)-methamphetamine; (3) unknown impurity.*

min under conditions identical to those for Fig. 1 but failed to show any enantiomer resolution. Clark and Barksdale also reported similar difficulties when attempting to separate amphetamine enantiomers using 1-[(4-nitrophenyl) sulfonyl] prolyl chloride (NPSP-Cl) as chiral derivatizing agent [*12*]. Figure 2 illustrates the chemical structures of the compounds examined, with the absolute configuration and optical rotation of each.

Figure 3 shows a racemic mixture of methamphetamine separated under reversed-phase conditions using THF:water (3:7). Confirmation of the elution order was made by derivatization of pure S-(+)-methamphetamine with GITC and chromatography under the same conditions. Figure 4 shows the chromatography of a sample submitted to this laboratory for analysis. Intelligence information indicated that the sample had been extracted from Vicks inhalers. Identification of the sample as methamphetamine was accomplished by infrared spectrophotometry (IR) with confirmation of the R-(−)-isomer by HPLC using GITC derivatization. Figure 5 illustrates chromatography of a liquid sample removed from a syringe found at the scene of a clandestine laboratory that was manufacturing methamphetamine. Figure 6 is the chromatography of a sample of powder purchased by an undercover police officer. The sample was identified by infrared spectrophotometry as containing methamphetamine and ephedrine. Methamphetamine was found to be present as the S-(+)-isomer, and ephedrine was found to be the R,S-(−)-isomer. The structure of methamphetamine-GITC is shown in Fig. 7.

Several methods are available to the forensic drug chemist for the identification of amphetamine isomers. One method used in this laboratory involves the formation of amphetamine salts with optically active mandelic acid and subsequent identification by IR spectroscopy [*13*]. The determination of the enantiomeric composition of amphetamine and methamphetamine has generally been accomplished by reactions with heavy metal salts and observation of the crystal formations by polarized light microscopy [*14*]. Interpretation of the crystals may be difficult for the untrained microscopist and may require mixing enantiomers to identify (+)- or (−)-enantiomers.

In most instances forensic chemists establish the identity of drugs by IR spectroscopy or mass spectrometry (MS). Once the identity of a drug is established, isomer confirmation may be accomplished by HPLC using GITC. Although resolution of the isomers of amphetamine was not possible using THF:water (3:7), isomer resolution was possible with methanol:water (1:1). Figure 8 shows the separation of the amphetamine-GITC diaste-

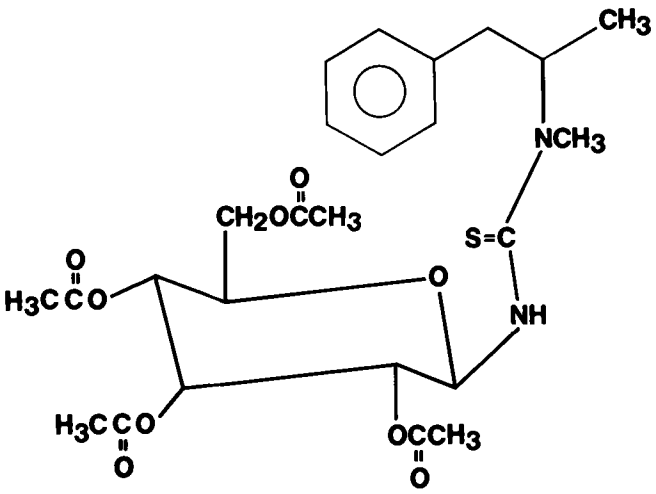


FIG. 7—*Structure of methamphetamine-GITC.*

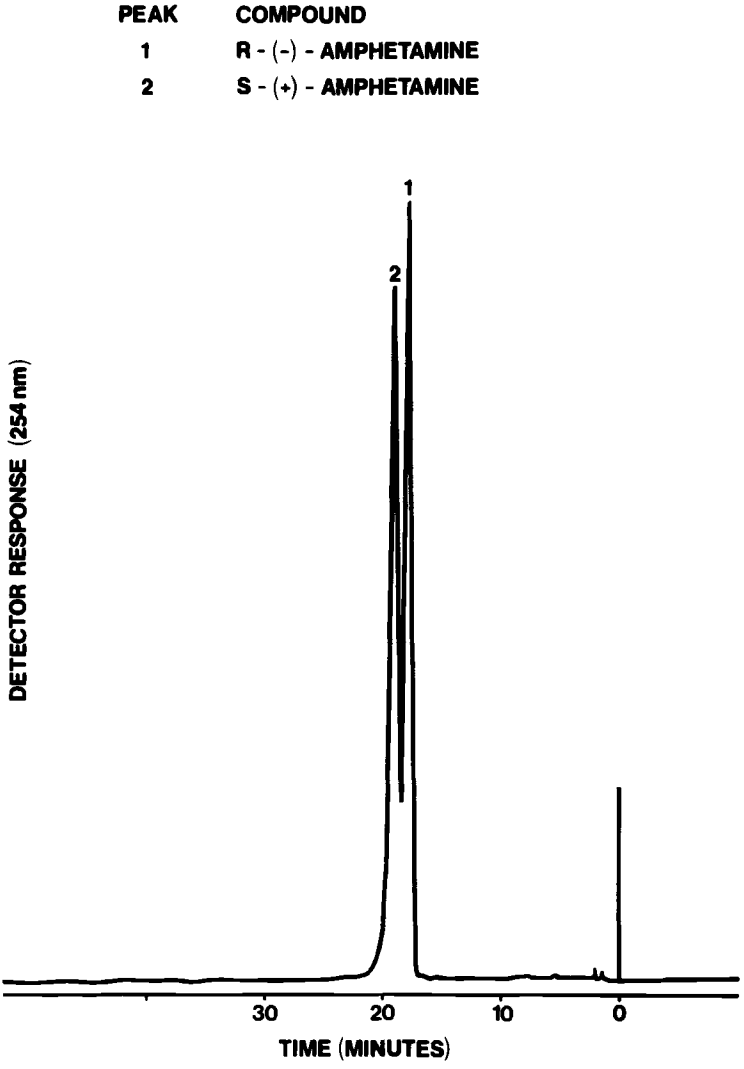


FIG. 8—*HPLC separation of GITC-derivatized racemic amphetamine using methanol: water (1 : 1) mobile phase. Peaks: (1) R-(−)-amphetamine; (2) S-(+)-amphetamine.*

reomers. The elution order of the amphetamine enantiomers is the R-(−)-isomer before the S-(+)-isomer.

GITC is a useful derivatizing agent for the determination of amine enantiomers in forensic sciences laboratories by reversed-phase HPLC using standard achiral stationary phases.

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