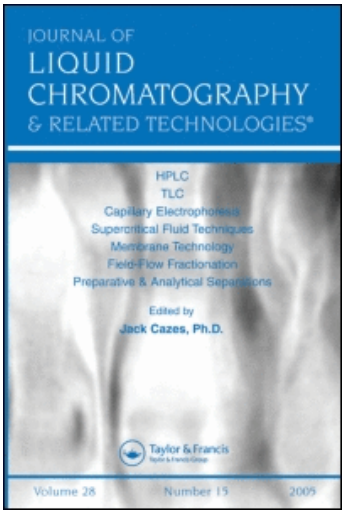

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LIQUID CHROMATOGRAPHIC ANALYSIS OF REGIOISOMERS AND ENANTIOMERS OF N-(CHLOROBENZYL)-α-METHYLPHENETHYLAMINES: ANALOGUES OF CLOBENZOREX

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ABSTRACT

The regioisomeric 2-, 3- and 4-chlorobenzylamphetamines were synthesized from racemic and (+)-amphetamine by reductive alkylation. The 2-, 3- and 4-chloro regioisomers were separated by reversed-phase liquid chromatography following phenylisothiocyanate derivatization. The indiviual enantiomers of each regioisomer were identified by HPLC following derivatization with GITC. Normal phase liquid chromatographic analysis of the diastereomeric GITC derivatives produced α-values of approximately 1.0 for each racemic pair of regioisomers. These methods were developed in order to specifically identify the drug clobenzorex, d-N-(2-chlorobenzyl)-α-methylphenethylamine and distinguish it from its optical and regioisomers.

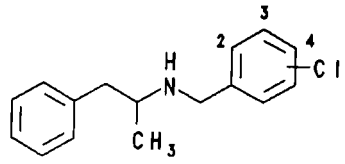
INTRODUCTION

Low molecular weight amines structurally related to amphetamine are present in a variety of formulations and street drug samples, and these compounds continue to be a challenging area of pharmaceutical and forensic analysis [1-5]. Designer drug modifications of many of the more established drugs of abuse have produced a variety of new compounds which differ only slightly in their structure in comparison to the parent compound [1-5]. Modifications such as homologation or regioisomerism produce compounds which often require the development of specific analytical methods and/or techniques for their specific identification [1-4]. Additionally, the analysis of street drug samples to determine the enantiomeric composition of the active drug ingredients can often distinguish a diverted pharmaceutical dosage form from a clandestinely manufactured substance [6,7].

The N-2-chlorobenzyl derivative of d-amphetamine, N-(2-chlorobenzyl)-α-methylphenethylamine or 2-chlorobenzylamphetamine, is marketed in Mexico and is known as clobenzorex. This drug is used in therapy for its anorexic properties which are similar to those of amphetamine. The d-enantiomers of amphetamine type compounds have higher stimulant and anorexic potencies than the enantiomeric l-isomer [8]. Clobenzorex has been encountered in forensic samples in recent years, requiring specific methods for its identification. These methods should be able to distinguish clobenzorex (2-chlorobenzyl) from its 3- and 4-chloro regioisomeric derivatives, as well as the enantiomeric composition of the sample.

In this study we have prepared the (+)-enantiomers and racemic mixtures of the three chloro regioisomers of

clobenzorex. The regioisomers were separated by reversed-phase liquid chromatography and the enantiomers were identified by normal phase chromatography following diastereomeric derivatization.



Clobenzorex (2-Cl) and its regioisomers

EXPERIMENTAL

Instrumentation. The liquid chromatograph consisted of a Waters Associates Model 6000A pump, U6K injector, Model 440 UV detector with dual wavelength accessory operated at 254, and a Houston Instruments OmniScribe dual pen recorder. Infrared spectra were recorded on a Perkin-Elmer Model 1710 Fourier transform infrared (FTIR) spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu Instruments Model UV-160 spectrophotometer. Nuclear magnetic resonance spectra (1H) were obtained using a Varian EM360 spectrometer. The electron impact mass spectra were obtained using a Hewlett-Packard 5970B mass selective detector. The ionization voltage was 70 eV and the source temperature was 220°C. The individual samples were dissolved in methanol (1 mg/mL) and 0.5 uL introduced into the mass spectrometer via the gas chromatograph equipped with a 12m x 0.31 mm id fused silica column with a 0.52 um thickness of OV-1. The column temperature was programmed from 70°C to 150°C at a rate of 15°C/min and from

150°C to 250°C at a rate of 25°C/min. The split ratio for the GC was 10:1 and all samples eluted in approximately 7 minutes.

Liquid Chromatographic Procedures. The reversed-phase analytical column was 30 cm x 3.9 mm id packed with uBondapak C_{18} (Waters Associates, Milford, MA). The analytical column was preceded by a 7cm x 2.1mm id guard column dry packed with CO:Pell ODS (Whatman). The amine-phenylisothiocyanate (PIT) derivatives were dissolved in HPLC grade methanol and chromatographed using a mobile phase of methanol, water and acetic acid (65:34:1). The mobile phase flow rate was 1.5 mL/min and the detector was operated at 1.0 AUFS.

The normal phase liquid chromatographic separations were accomplished using a 30 cm x 3.9 mm id u-Porasil (Waters Associates). The mobile phase was n-hexane, chloroform and 2-propanol (500:25:3) at 1.5 mL/min and the detector was operated at 254 nm and 0.2 AUFS.

Derivatization Procedures. PIT derivatives were prepared by dissolving 2 mg of the amine hydrochlorides in 30 mL of 0.45 N NaOH and extracting the free bases into 30 mL of chloroform. To this chloroform solution was added 10 uL of neat phenylisothiocyanate (PIT), and the reaction mixture evaporated to dryness under a stream of air. The resulting residue was dissolved in 1 mL of methanol and 5 uL of this solution was injected into the liquid chromatograph.

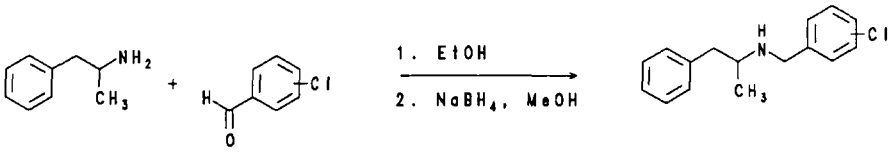
The GITC derivatives were prepared by first extracting the free bases into chloroform as described above, and then adding a 10% molar excess of GITC as a chloroform solution. After 10 minutes at room temperature this reaction mixture was evaporated

to dryness under a stream of air. The resulting residue was dissolved in 1 mL of chloroform and 5 uL of this solution injected into the liquid chromatograph.

Synthesis of the Clobenzorex Derivatives. Racemic or (+)-amphetamine sulfate (685 mg, 1.86 mmol) was dissolved in water (25 mL) and the solution made basic (pH 12) with a saturated solution of NaOH. The basic aqueous solution was extracted with ether (3 X 25 mL) and the combined ether extracts dried over $MgSO_4$, filtered and the filtrate evaporated in vacuo to obtain the free base. The base was dissolved in absolute ethanol (20 mL), the appropriate chlorobenzaldehyde added (540 mg, 3.84 mmol) and the reaction mixture stirred at room temp for 18 - 20 hours. The ethanol solvent was then removed in vacuo to yield the intermediate imines as viscous yellow oils. The crude imines were dissolved in methanol (25 mL) and $NaBH_4$ (130 mg, 3.5 mmol) was added portionwise. The reaction mixture was then stirred at room temp for 2 hours, then at reflux for 1 hour. After cooling (room temp), the mixture was evaporated in vacuo and the residue suspended in water (25 mL). The aqueous suspension was acidified (pH 1) with dilute HCl, and then made alkaline (pH 12) with dilute NaOH. The basic suspension was then extracted with ether (3 X 25 mL) and the combined ether extracts dried ($MgSO_4$), filtered and the filtrate evaporated in vacuo to yield the product bases as pale yellow oils. Treatment of the bases with ethereal HCl afforded the hydrochloride salts which were isolated by filtration.

RESULTS AND DISCUSSION

The regioisomeric 2-, 3- and 4-chlorobenzylamphetamines were sythesized from racemic and (+)-amphetamine by reductive alkyla-



Scheme I. Synthesis of the regioisomeric N-(chlorobenzyl)-α-methylphenethylamines.

tion as outlined in Scheme I [1,2]. Treatment of the free base of amphetamine with commercially available chlorobenzaldehydes yielded the intermediate imines. The crude imines were then reduced to the desired regioisomeric and enantiomeric chlorobenzylamphetamines with sodium borohydride in methanol. The bases of these compounds were converted to the corresponding hydrochloride salts with etheral hydrochloric acid. The structures of all products were confirmed by ultraviolet, infrared, proton NMR and mass spectral analysis.

The liquid chromatographic retention properties of clobenzorex and its regioisomers were quite similar under a variety of conditions. The underivatized amines were examined by reversed-phase techniques on both C_{18} and phenyl stationary phases with only limited success. The 2-chloro regiomer (clobenzorex) was well resolved in several systems, however the 3- and 4- isomers coeluted in all cases. Therefore, the amines were derivatized with phenylisothiocyanate (PIT) to form the phenylthiourea derivatives in an effort to improve the chromatographic properties of these compounds [9]. The isocratic reversed-phase separation of the PIT derivatives in Figure 1 was obtained with a C_{18} stationary phase and a mobile phase of methanol, water and acetic acid (65:34:1). The 3-chloro isomer has the

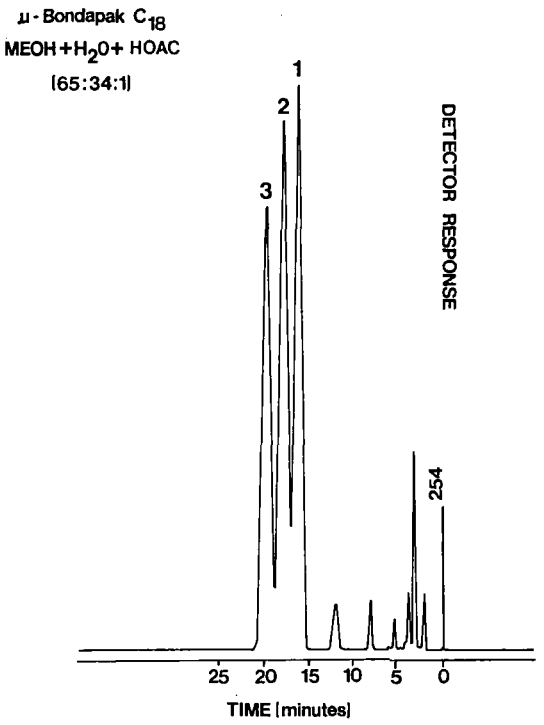
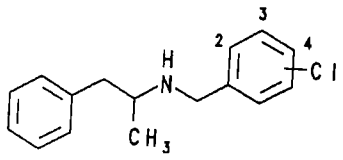


Figure 1. Reversed-phase liquid chromatographic separation of N-(chlorobenzyl)-α-methylphenethlyamines. Peaks: 1 = 3-chloro; 2 = 4-chloro and 3 = 2-chloro.

lowest capacity factor in this system followed by the 4-isomer, and clobenzorex (2-chloro isomer) has the highest capacity factor.

The underivatized amines were also examined by gas chromatography and the results were similar to those obtained by HPLC. The GC system was an OV-1 capillary column (12 m X 0.31 mm id) and a temperature gradient from 70°C to 250°C. The 3- and 4-chloro regiomers were unresolved in this and other variations of the temperature program.

Table 1. Yields and melting points of the chlorobenzorex regioisomers.



Amine	Stereochem	Yield, % (base)	mp, °C (HCl)
2-Chloro	(+)	83	174-176[a]
3-Chloro	(+)	81	145-147
4-Chloro	(+)	79	203-204
2-Chloro	(±)	89	169-170
3-Chloro	(±)	93	164-164
4-Chloro	(±)	70	184-186

[a]The literature melting point for (+)-clobenzorex is 182 - 183°C.

Since the pharmaceutical dosage form of clobenzorex contains only the (+)-enantiomer, it is important to determine the enantiomeric composition of the sample. A variety of methods for the chiral separation of various drugs have been reported [10-14]. Most of these are liquid chromatographic methods making use of chiral stationary phases, chiral mobile phase additives or precolumn diastereomeric derivatization. In many applications, the chemical derivatization of the enantiomeric amines with chiral reagents to yield diastereomeric products offers the advantage of speed and convenience. Many chiral derivatizing agents are commercially available and the resulting diastereomeric products can

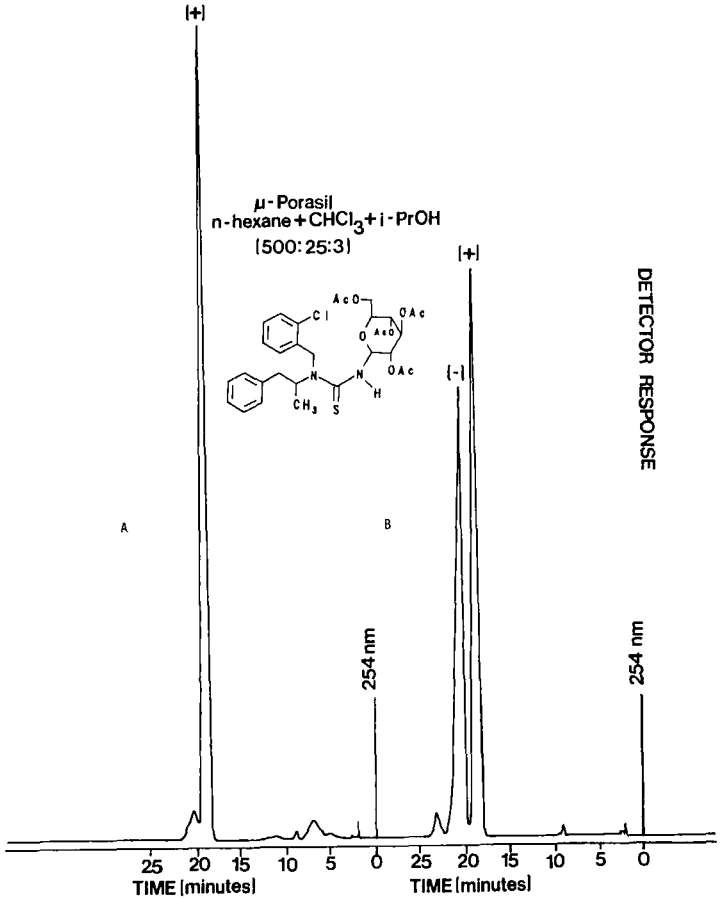


Figure 2. Normal-phase liquid chromatographic separation of GITC-derivatized enantiomers of N-(2-chlorobenzyl)-α-methylphenethylamine. A = (+)-enantiomer; B = racemic mixture.

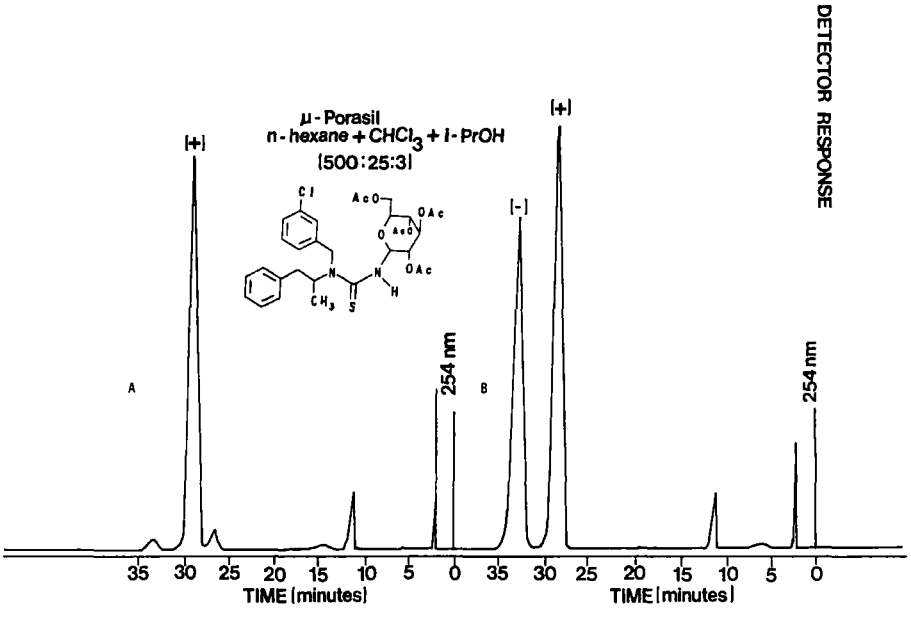


Figure 3. Normal-phase liquid chromatographic separation of GITC-derivatized enantiomers of N-(3-chlorobenzyl)-α-methylphenethylamine. A = (+)-enantiomer; B = racemic mixture.

be resolved on standard achiral stationary phases. Thus, the chiral analysis of a sample requires only the additional chiral derivatizing agent. One such agent, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), has been used in forensic analysis for the derivatization of chiral drugs of abuse which are primary and secondary amines [6,7,15]. The derivatization of clobenzorex and its regioisomers was accomplished in chloroform solution at room temperature using a slight excess of GITC. The resulting thiourea diastereomers were separated by normal-phase chromatography on a silica column (μ-Porasil) and a mobile phase

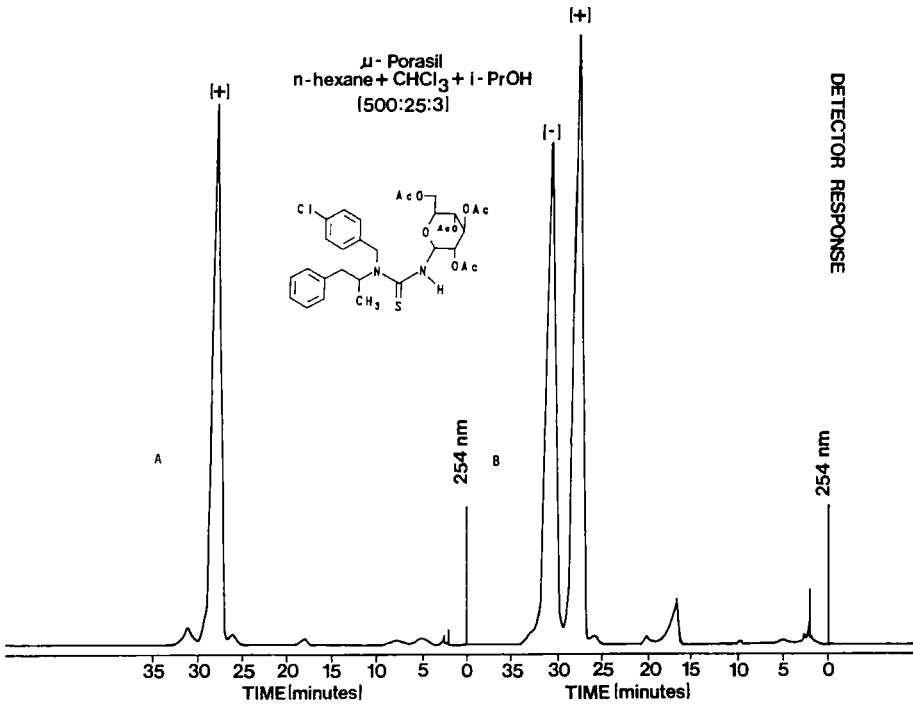


Figure 4. Normal-phase liquid chromatographic separation of GITC-derivatized enantiomers of N-(4-chlorobenzyl)-α-methylphenethylamine. A = (+)-enantiomer; B = racemic mixture.

of n-hexane, chloroform and isopropanol (500:25:3). Figures 2 - 4 show the GITC-derivatized (+)-isomers (Figures 2A, 3A and 4A) and the racemic mixtures (Figures 2B, 3B and 4B) for the 2-, 3- and 4-regiosiomeric N-(chlorobenzyl)-α-methylphenethylamines. In each case the A-chromatogram shows the regioisomer prepared from (+)-amphetamine to be predominately the (+)-enantiomer with perhaps a trace amount of the other enantiomer. This small amount of the other enantiomer may be due to enantiomeric contamination

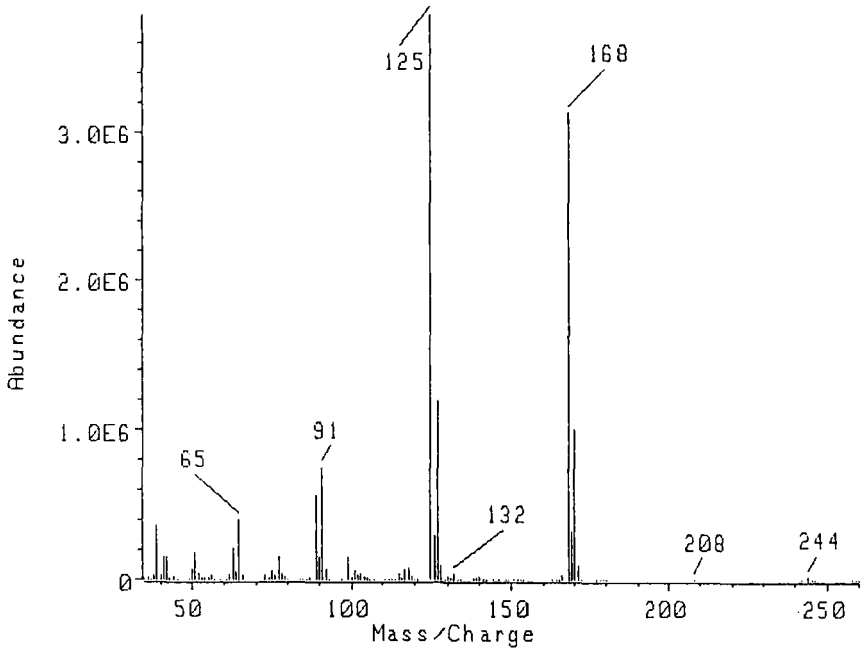
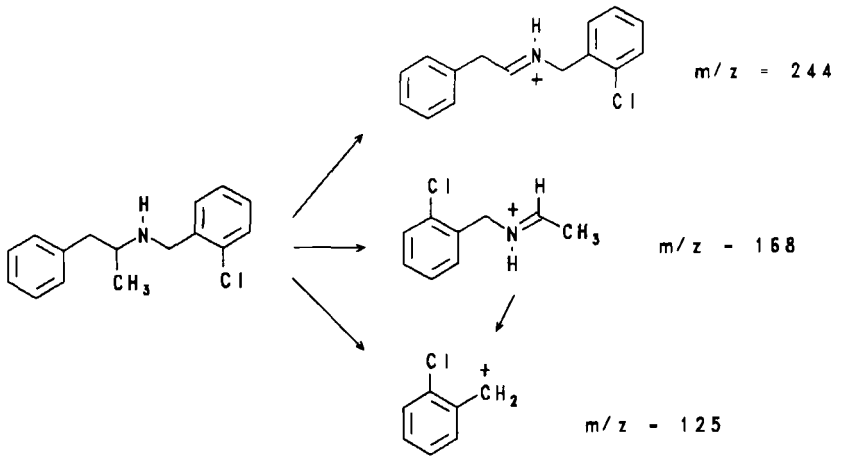


Figure 5. Mass spectrum of N-(2-chlorobenzyl)-α-methylphenethylamine.

of either the original amphetamine starting material or the GITC derivatizing reagent. The goal in most forensic analyses is not to determine the exact quantitative enantiomeric composition of a sample, but whether it consists primarily of one enantiomer or is a racemic mixture. Thus the presence of small amounts of the other enantiomers does not pose a significant problem in these analyses. In Figures 2B, 3B and 4B, the racemic amines are separated with near baseline resolution in each case. The (+)-enantiomer has the lower capacity factor in each case. These GITC-amine derivatives were not well resolved by reversed-phase methods. The combined use of derivatization methods (both PIT



Scheme II. Mass spectral fragmentation pattern for the N-(chlorobenzyl)-α-methylphenethylamines.

and GITC) allows for the analysis of N-(chlorobenzyl)-α-methylphenethylamines for both positional and enantiomeric isomerism.

The mass spectrum of clobenzorex (the d-(+)-2-chloro regioisomer) is shown in Figure 5. The mass spectra of all isomers were identical since fragmentation is generally unaffected by such isomerism. The mass spectra were obtained in a GC-MS system using an OV-1 column with temperature programming. The base peak in the EI spectrum occurs at m/z 125 and is likely the chlorobenzyl fragment as shown in Scheme II. The other major fragment in the mass spectrum occurs from the loss of the unsubstituted benzyl radical from the alpha-cleavage reaction of the amino moiety. Thus, the mass spectrum confirms the N-(chlorobenzyl)-amphetamine structure, while the HPLC methods determine the position of the chloro substituent and the enantiomeric composition of the sample.

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