A Rapid Differentiation of Pseudoephedrine and Ephedrine by Stereospecific Derivatization

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ABSTRACT: Pseudoephedrine and ephedrine are two common starting components in the illicit production of methamphetamine. Crime laboratory analysts have a need for a rapid, effective, and straightforward means of differentiating these two compounds. This presentation provides reaction pathways and analytical parameters related to the analysis of these precursors and subsequent derivatives. Derivatives are formed using a stereospecific derivatization involving N-Methyl-N-(trimethylsilyl) trifluoroacetamide and (S)-(t)-a-methoxy-a-trifluoromethylphenylacetyl chloride. Gas chromatography mass spectrometry data is presented with a discussion of separation efficiency and assessment of the potential this technique has for use in an accredited crime laboratory.

KEYWORDS: Stereospecific derivatization, chiral, enantiomer, diastereomer, pseudoephedrine, ephedrine, GC-MS, MSTFA, MTPACl

The object of this project was to develop a method to differentiate between the diastereomers, ephedrine and pseudoephedrine, as well as the enantiomers (+) and (-) pseudoephedrine. Both enantiomers and diastereomers are stereoisomers, molecules with the same chemical formula, but a different three-dimensional arrangement. An enantiomer is a non-superimposable mirror-image compound, such as (+) and (-) pseudoephedrine. A diastereomer is a non-mirror image stereoisomer, such as ephedrine and pseudoephedrine which have different attachment points at two chiral centers. The diagram below demonstrates these differences:

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Compounds that are stereoisomers of each other cannot be differentiated in their original forms by instrumentation commonly used for analysis. Pseudoephedrine and ephedrine have identical mass spectra and gas chromatograms. See figures 1-4.
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Figure 1: Gas Chromatogram of Ephedrine

![Ephedrine Chromatogram](image1)

Note the similar retention times of ephedrine (1.12 min.) and pseudoephedrine (1.13 min.). These two compounds are undistinguishable in their underivatized forms. The 2.07 peak in ephedrine is a result of the impurity Guafessin, an expectorant found in cough syrup that is not easily removed from the ephedrine extract.

Figure 2: Gas Chromatogram of Pseudoephedrine

![Pseudoephedrine Chromatogram](image2)
In the justice system, there is a need for the rapid differentiation of these two compounds because they are both precursors to the drug methamphetamine. Ephedrine is listed as a schedule II drug by the DEA; a larger yield of methamphetamine can be produced from ephedrine. Pseudoephedrine is available over the counter in cold medicine. It is not listed on the DEA drug schedule. These factors lead to additional jail time for methamphetamine producers using ephedrine as a precursor; thus the prosecution impetus for rapid differentiation.
After the derivatization procedure is performed, the resulting compound can be analyzed using gas chromatography-mass spectrometry (GC-MS). The gas chromatography portion of the instrument breaks a sample up into fragments and separates them by mass. The mass spectrometer determines the molecular weight of the fragments and is able to identify them using a computer database. The following derivatization procedure is adapted from Donike and Shin\(^3\). Their data showed separation achieved on a Seeborn 54 column. GC-MS parameters were adapted to work on an HP-1 15m column, which is common in crime laboratories. The run time was also shortened.

**Materials and Methods**

**Reagents**

- (1R,2R)-(-)-Pseudoephedrine 98% Aldrich
  - Lot #04009KO
- (1S,2S)-(+)-Pseudoephedrine 98% Aldrich
  - Lot # 14408PO
- Ephedrine II Nasal Decongestant and Expectorant 25mg ephedrine hydrochloride with guaifessin
- Non-drowsy Top Care Maximum Strength
  - 12-hour decongestant 120mg pseudoephedrine hydrochloride
- N-Methyl-N (trimethylsilyl) trifluoroacetamide (MSTFA), Sigma Lot # 033K1128
- (S)-(+) -α-Methoxy-α-(trifluoromethyl) phenylacetyl chloride (MTPACl) 99%, Aldrich Lot # 07729TB

**Equipment**

- Fischer Scientific-Isotemp Oven
- GC-MS GC-17A Gas Chromatograph, Shimadzu
  - HP-1, 15m column

**Extraction Procedure:**

Ground pseudoephedrine or ephedrine tablets (~0.006g) are added to a centrifuge tube and dissolved with 1M H\(_2\)SO\(_4\). Some binder remains visible in the solution. An equal volume 1-chlorobutane is added to the tube. The mixture is centrifuged and vortexed for five minutes. The top (1-chlorobutane) layer is taken off with a Pasteur filter pipette and discarded. The solution is made basic to pH 10 by the addition of NaCO\(_3\) solid. Basicity is confirmed with pH paper. An equal volume of 1-chlorobutane is added. The vortexing and centrifugation is repeated. The top layer is removed and evaporated gently under N\(_2\) with no heating. A white solid results. Continued presence of impurities in the extracted pseudoephedrine led us to purchase (-) and (+) pseudoephedrine. The following data was obtained using extracted ephedrine and purchased pseudoephedrine.

**Derivatization Procedure:**

A solvent mixture of 60:40 (v/v) acetonitrile and trifluoroacetic acid was prepared. 0.002g methyl orange solid was added. Extracted material was dissolved in 50 microliters of the above mixture. 140 microliters MSTFA was added. This changes the reaction mixture from red to yellow. The mixture is heated in an 110°C oven for five minutes. Five microliters of MTPACl are added and the mixture is heated for another five minutes. The resulting mixture is evaporated under nitrogen. The reaction scheme for the derivatization is shown below.
GC-MS Parameters:
Derivatized material is dissolved in methylene chloride and analyzed using GC-MS.
Sampling Time: 1 min.
Injection Temperature: 275°C
Interface Temperature: 320°C
Carrier Gas Helium
Inlet Column Pressure 2kPa
Column Flow 0.7 mL/min
Split Ratio 70
Total Flow 50 mL/min
Carrier Flow 50mL/min
Splitless

Results and Discussion:
Figure 5 shows GC-MS data from a SIM (selected ion monitoring) program. SIM at 438 allows for detection of the derivatized product. The data shows resolution of (-) and (+) pseudoephedrine peaks at 7.093 min and 7.175 min (+) respectively. Figure 6 shows the separation of (-) and (+) pseudoephedrine enantiomers as well as their diastereomer, ephedrine.
Figure 5: GC-MS Data Showing (-) (+) Pseudoephedrine Differentiation

Figure 6: Differentiation of Pseudoephedrine and Ephedrine
Conclusions:

The above data shows that pseudoephedrine and ephedrine have been effectively derivatized. The derivatized products can be resolved using an HP-1 column. Further steps include derivatizing a mix of ephedrine enantiomers and validating the procedure for implementation in an ASCLD certified crime laboratory.

References:

3 Shin, Ho-Sang; Donike, Manfred. Stereospecific Derivatization of Amphetamines, Phenol Alkylamines, and Hydroxyamines and Quantification of the Enantiomers by Capillary GC/MS. Analytical Chemistry 1996; 3015-3020.

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