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FRACTIONATION AND GAS CHROMATOGRAPHIC ANALYSIS OF AZA-ARENES IN COMPLEX MIXTURES

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SUMMARY

Complex mixtures of nitrogen-containing polyaromatics (aza-arenes) require fractionation for improved resolution and trace component enrichment prior to gas chromatographic–mass spectrometric (GC–MS) identification. Suitability of high-performance liquid chromatography using a polar stationary phase to meet this goal is demonstrated. Evaluation of stationary phases for the separation of complex aza-arene mixtures by capillary GC was carried out using selectivity, thermal stability, and column deactivation as criteria. Separations up to 5-ring structures are subsequently demonstrated, using the basic fraction of cannabis smoke condensate and coal tar as examples.

INTRODUCTION

Compositional studies of various products of combustion with regard to polycyclic aromatic compounds (PACs) have lately been both popular and necessary. With recent world-wide projections of a wider utilization of coal-derived products, environmental concerns about this generally toxic and carcinogenic class of compounds have frequently been voiced. In addition, a steadily increasing habit of smoking tobacco (and, more recently, cannabis) needs improved analytical methodology as well as biological testing.

Numerous analytical approaches to PACs exist today[1] in which high-efficiency chromatographic techniques assume an important role. While fairly adequate methodology is available for neutral PACs in terms of chromatographic separation and identification efforts[2–7], this situation is not yet paralleled for nitrogen-, sulfur-, or oxygen-containing PACs. Yet, ample evidence suggest high toxicity of certain of such compounds. Studies have been underway in our laboratories to address these problems; the work reported to date in this area concerns isolation and chromato-

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graphy of sulfur PACs[8] and capillary gas chromatographic–mass spectrometric (GC–MS) methods for nitrogen aromatic compounds in complex mixtures[9]. In the latter publication, importance of both high-precision retention data and mass spectral information was emphasized. The present study extends considerably the range of studied compounds and addresses the problem of sample fractionation needed for reliable determination of trace mixture components.

The previous communication[9] emphasized the importance of deactivation of glass capillary columns to chromatographing nitrogen aromatics as highly symmetrical peaks, but the available columns could elute such compounds only up to 3-ring structures. While *non-polar* glass capillary columns are of sufficient inertness due to the recently advanced column technologies, they do not offer adequate resolution of complex aza-arene mixtures; certain column selectivity is required in this case. A polyglycol phase recently described by Verzele and Sandra[10] as well as polyimide phases were evaluated for separation of aza-arenes up to 5-ring structures in this work.

Just as in the case of neutral PACs[2–4], fractionation of a complex mixture is essential to the enrichment of trace components prior to GC–MS determination. High-performance liquid chromatography (HPLC) with an aminosilane-bonded phase is shown to meet this goal for some complex mixtures of nitrogen aromatics. While the methodological aspects are emphasized in this study, the method was used to characterize nearly 300 nitrogen compounds in the basic fraction of cannabis smoke condensate[11].

EXPERIMENTAL

Capillary gas chromatography

A modified Varian Model 1400 gas chromatograph was employed throughout this work. Different glass capillary columns were used to cover an extensive volatility range of various nitrogen compounds. Submicroliter amounts of samples dissolved in an appropriate solvent (typically, methylene chloride) were injected directly into the capillary columns.

While glass capillary columns coated statically with UCON 50-HB-2000 (a polypropylene glycol) and using tetraphenylboron sodium as a deactivation agent were prepared as described previously[9], an optimized column technology had to be developed for heavier aza-arenes. The previously described polyimide stationary phases[12] were coated statically inside glass capillary columns after different surface treatments; satisfactory, but not outstanding columns resulted.

Columns with good efficiency and deactivation were prepared with the Superox-4 stationary phase (available from Alltech, Arlington Heights, IL, U.S.A.). Different surface treatments were evaluated with this phase, while the final columns were tested for efficiency, thermal stability, and deactivation using standard mixtures of pyridine and quinoline bases injected in nanogram quantities. Since this stationary phase forms a very viscous solution, the columns must be filled under high pressure prior to the usual static coating[13].

Glass capillary columns coated with OV-101 non-polar silicone polymer (obtained from Applied Science Labs., State College, PA, U.S.A.) were first leached with an acid and silylated[14] as previously described[15].

Preparation of samples and their HPLC fractionation

Smoke condensate was obtained from 300 Mexican marijuana cigarettes by means of a smoking machine. Puffs of 2-sec duration at 1-min intervals were applied, and smoke was trapped in acetone using a cryogenic trap held at approximately −60°C.

The smoke condensate was fractionated according to a previously described scheme[2,16]. The basic fraction obtained through pH and solubility manipulations[2] has been verified to contain only nitrogen compounds in a previous study[9].

Similar fractionation was applied to a sample of crude coal tar and the isolated basic fraction was chromatographed up to 260°C on a Superox-4 capillary column. A previous characterization of various coal tar fractions in this laboratory[17] yielded also information on pyridine and quinoline bases. However, hydrogen-donor molecules, such as substituted indoles, carbazols and benzocarbazols, do not partition into the basic fraction[17]. Although GC–MS identification of the coal tar bases separated on Superox-4 remains to be completed, existence of up to 5-ring structures is tentatively suggested from retention data.

HPLC fractionations of marijuana smoke bases was accomplished using four 90 cm × 1.0 mm I.D. columns (connected in series), dry-packed with an aminosilane treated siliceous packing (Porasil C, 37–75 μm, obtained from Waters Assoc., Milford, MA, U.S.A.). Porasil C was first reacted with 10% solution of 3-(2-aminoethylamino)propyl trimethoxysilane in toluene and the final packing was successively washed with solvents of different polarity. Successive injections of marijuana smoke bases were used to pre-concentrate enough material for GC–MS investigations. While this communication concentrates on the methodological aspects, detailed identifications of marijuana smoke bases will be reported elsewhere[11].

RESULTS AND DISCUSSION

Mixtures obtained under various conditions of combustion are extremely complex. Just as with neutral PACs, aza-arenes as a group of compounds, give rise to numerous isomers due to a variety of ring fusion and substitutions. Even with the best available separation methods, it is exceedingly difficult to separate various PACs from each other and ascertain reliable determinations of selected compounds. If nitrogen compounds are present in sufficient quantities, specificity of their determination can be secured without mixture fractionation by a nitrogen-sensitive detector[18,19] in the presence of other molecular species.

Sample fractionation is often required to (a) remove compounds that might interfere with analysis; and (b) enrich the trace components for identification or quantitation purposes. The fractionation scheme[2,16] developed initially for concentration of neutral PACs, also yields a fraction of nitrogen bases in one of the initial steps. The bases are simply partitioned into an aqueous acid layer, and later re-extracted in their free form into methylene chloride at an elevated pH. This method was used successfully to isolate the basic fractions of solvent-refined coal[9], coal tar[17] and tobacco and marijuana smoke[11]. With one of these samples[9], the specificity of this fractionation was ascertained through a peak-by-peak agreement between the flame-ionization and nitrogen-sensitive detectors. On the other hand, hydrogen-donor type nitrogen-containing compounds do not partition with the rest of aza-arenes[17].

Typical complexity of a fraction of nitrogen compounds obtained through the simple partition process is demonstrated in Fig. 1; the sample originates from marijuana smoke condensate subjected to the partition scheme[2]. Although this chromatogram extends only to 3-ring structures, difficulties were encountered in identification studies by GC–MS, as overlapping peaks often prevented reliable identification. In addition, trace components could not be recorded due to the overall limited capacity of a capillary column. The HPLC fractionation method, as demonstrated here, largely overcomes these difficulties.

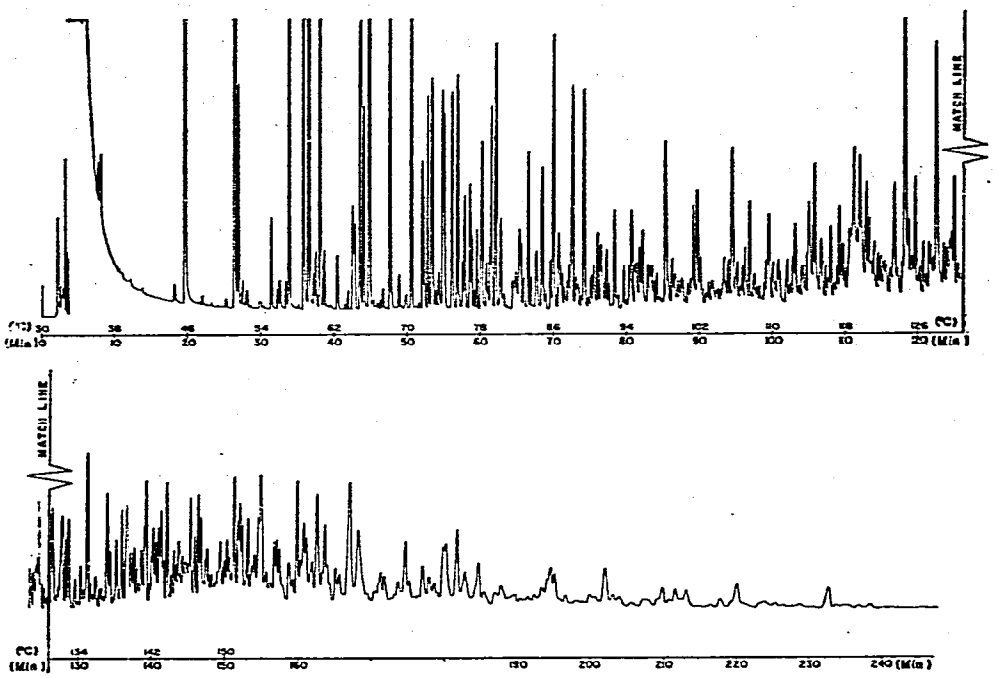

Fig. 1. Chromatographic separation of a concentrate of the basic fraction of cannabis smoke condensate. Column: 50 m × 0.25 mm I.D. glass capillary column coated with UCON 50-HB-2000. Most tentatively identified components belong to the following compound classes: alkylated pyridines, pyrazines, alkoxypyridines, acylpyridines, aminopyridines, substituted imidazoles, indazoles, substituted quinolines, pyrimidines, and various amides.

While Fig. 1 clearly indicates the general sample complexity and many overlaps, partially resolved peaks, shoulders, etc., a considerable improvement of this situation is seen in Fig. 2. In our case, the fact that HPLC fractionates the mixture according to polarity (while it is still *primarily* according to volatility in capillary GC) is highly beneficial; only limited overlap between the two HPLC subfractions exists, which makes the identification task easier.

The stationary-phase selectivity combined with adequate deactivation and thermal stability of the columns are needed for adequate resolution and reliable analyses of aza-arenes at the level of nanograms and below. In order to prepare such columns, acid leaching[15] of glass surface ingredients prior to coating was found

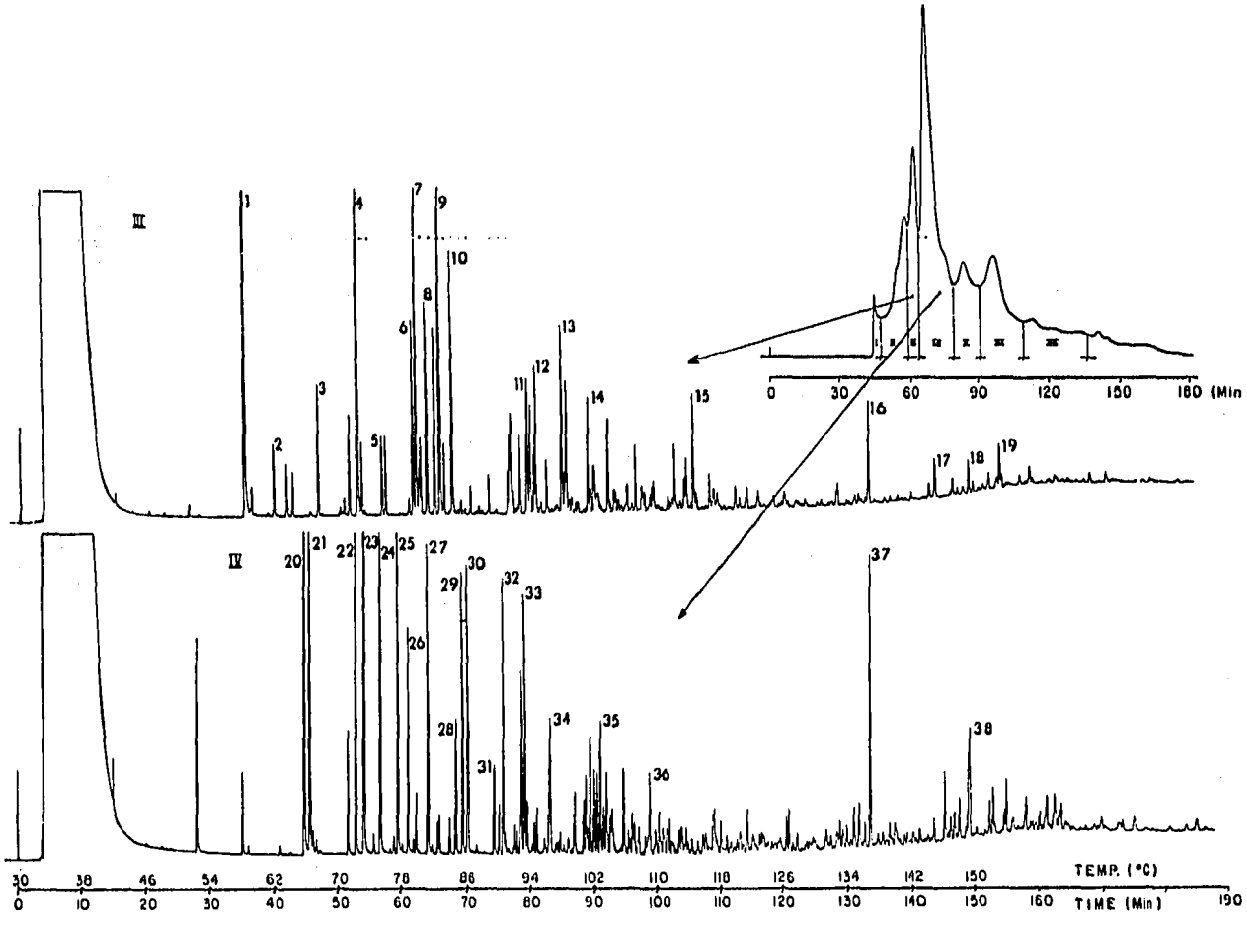

Fig. 2. HPLC fractionation of the basic fraction of cannabis smoke. Conditions: mobile phase, *n*-hexane, flow-rate 0.5 ml/min; detector, Perkin-Elmer LC-55 variable-wavelength monitor, setting at 254 nm; capillary GC column: as in Fig. 1; fractions III and IV from the HPLC column were concentrated and injected directly onto the glass capillary column. Peaks: 1 = 2-methylpyridine; 2 = methylpyrazine; 3 = 2-ethylpyridine; 4 = 2,5-dimethylpyridine; 5 = 2,3-dimethylpyridine; 6 = 2-vinylpyridine; 7 = a trimethylpyridine; 8 = a trimethylpyridine; 9 = a methyl-2-ethylpyridine; 10 = a methyl-3-ethylpyridine; 11 = a methyl-2-propylpyridine; 12 = a methylvinyl- or propenylpyridine; 13 = a methyl-2-isobutyl (?) pyridine; 14 = N-furfurylpyrrolidine; 15 = a methyl-2-pentylpyridine; 16 = 2-methylquinoline; 17 = 2,4-dimethylquinoline; 18 = a dimethyl- or ethylquinoline or -isoquinoline; 19 = a dimethyl- or ethylquinoline or -isoquinoline; 20 = 3-methylpyridine; 21 = 4-methylpyridine; 22 = 2,5-dimethylpyridine; 23 = 2,4-dimethylpyridine; 24 = 2,3-dimethylpyridine; 25 = 3-ethylpyridine; 26 = 4-ethylpyridine; 27 = 3,5-dimethylpyridine; 28 = a methyl-3-ethylpyridine; 29 = 2,4,6-trimethylpyridine; 30 = 3-vinylpyridine; 31 = 3-propylpyridine; 32 = a trimethylpyridine; 33 = a trimethylpyridine; 34 = a methyl-4-ethylpyridine; 35 = a methylvinylpyridine; 36 = a dimethyl- or ethylvinylpyridine; 37 = 2-methylquinoline; 38 = a dimethyl- or ethylquinoline or -isoquinoline.

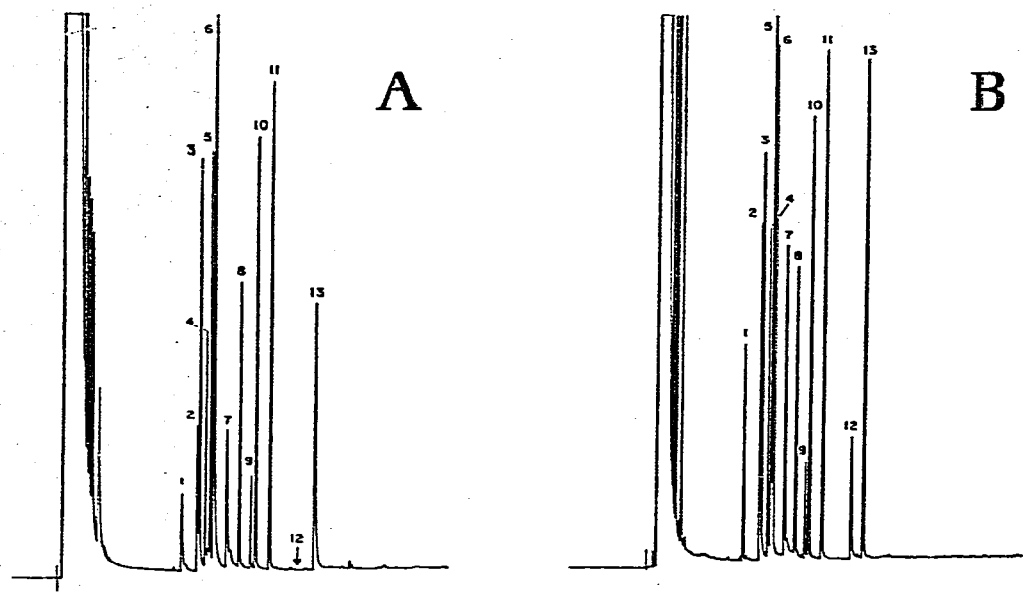

Fig. 3. Chromatograms of a standard mixture of two Superox-4 glass capillary columns with different surface treatment: (A) soda-lime glass without pretreatment; and (B) soda-lime glass, leached[15] with formic acid. (Benzyl)triphenylphosphonium chloride was added to the stationary-phase solution in both cases; column dimensions: 16 m × 0.26 mm I.D. Standard mixture: 1 = 2-aminopyridine; 2 = 2-amino-6-methylpyridine; 3 = quinoline; 4 = 2-amino-5-methylpyridine; 5 = isoquinoline; 6 = 8-methylquinoline; 7 = 2-amino-4-methylpyridine; 8 = 7-methylquinoline; 9 = unknown; 10 = 4-methylquinoline; 11 = 2,4-dimethylquinoline; 12 = 2,2′-bipyridyl; 13 = 4-phenylpyridine.

essential. Further deactivation is provided through treatment with (benzyl)triphenylphosphonium chloride, while Superox-4 columns with tetraphenylboron sodium addition had inferior thermal stabilities. Peak shapes for the model mixture (as seen in Fig. 3) are affected substantially by a column pretreatment technique. Note particularly the cases of 2-aminopyridine and bipyridyl (peaks 1 and 12).

Stationary-phase selectivity in capillary GC of aza-arene mixtures appears desirable. While analyzing complex mixtures of such compounds with non-polar (methylsilicone) columns, we experienced resolution problems even with the best high-efficiency columns of this kind. In contrast, more polar columns (such as those coated with polyimides or Superox) tend to yield improvement in resolution. Illustration of an improved isomer resolution is shown in Fig. 4 *versus* 5, where compounds III and IV are clearly resolved with the Superox-4 column, but not with the OV-101 silicone phase.

For Superox columns, thermal stability appeared adversely influenced by the presence of oxides in glass surface. This is in agreement with the earlier published observations by Schomburg *et al.*[20] and ourselves[15] that concerned other types of stationary phases; depolymerization and a consequent phase "bleeding" have been suggested as explanation[20]. Thermal stability of the Superox columns is adequate up to at least 5-ring aza-arene structures (see Fig. 4), while improved resolution of the corresponding complex mixtures is also available.

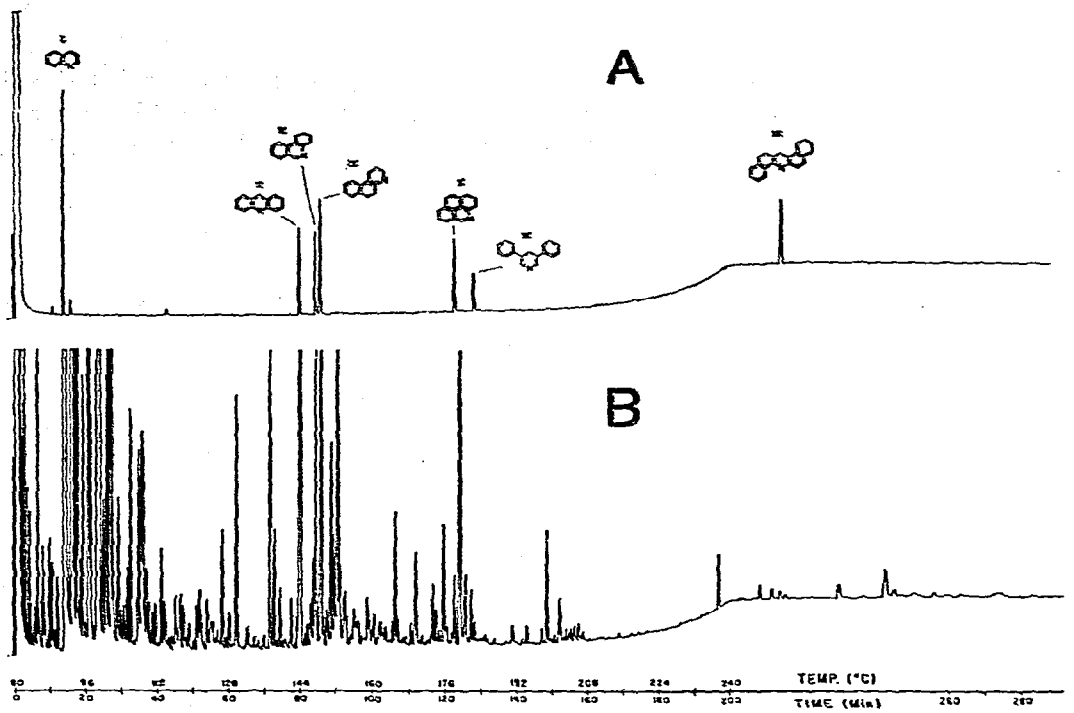

Fig. 4. High-temperature separations of aza-arenes on a 16 m × 0.26 mm I.D., Superox-4 glass capillary column. A = standard aza-arene mixture: I = quinoline; II = acridine; III = phenanthridine; IV = benzo[*f*]quinoline; V = 4-aza-pyrene; VI = 3,5-diphenylpyridine; and VII = 1,2,5,-6-dibenzoacridine. B = basic fraction of crude coal tar.

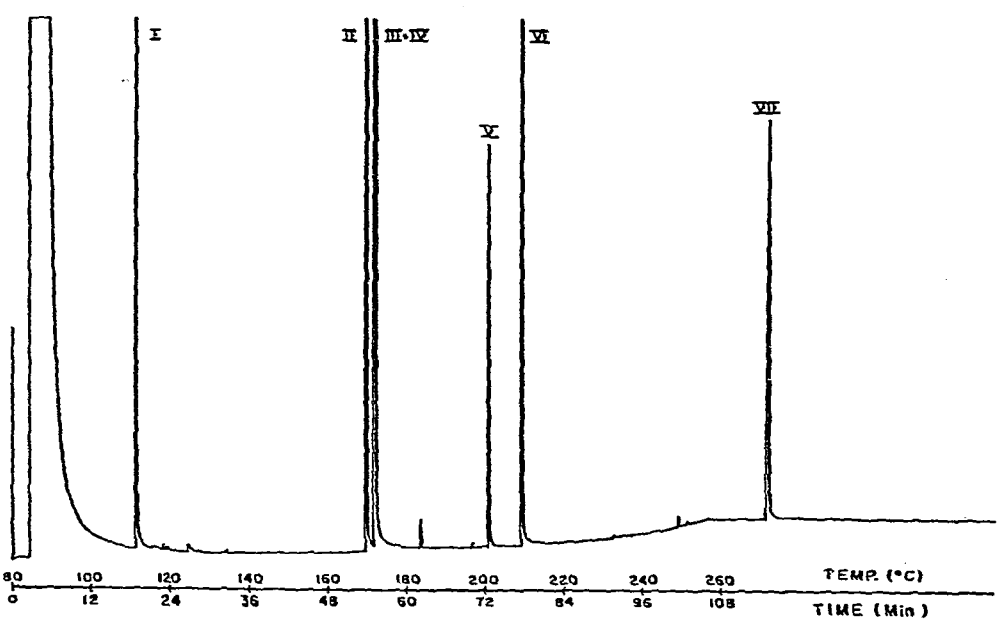

Fig. 5. Chromatogram of standard aza-arene mixture (same composition as in Fig. 4) on a 50 m × 0.25 mm I.D. glass capillary column coated with OV-101 methyl silicone phase.

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