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Review

Chromatographic determination of benz[*c*]acridines and related compounds in airborne carcinogens

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Abstract

Carcinogenic benzacridines from air, ground, and water pollution are determined by a variety of chromatographic methods. In all, five different chromatographic modes were in some cases used consecutively.

Contents

1. Introduction

Air pollution has been increasingly associated with the rise of many types of cancer in man. A study of air pollution and the sources of man-made pollutants seem to indicate that there is a disproportionate rise in the incidence of cancers in urban population groups [1,2]. Various polycyclic aromatic hydrocarbons, their alkyl derivatives, and their azaheterocyclic analogues, including benzacridines, are well-known carcinogens.

Determination of benz[*c*]acridine (Fig. 1, **1**) and related compounds (azaarenes) is very important because of their known carcinogenicity. They have been found in many different environ-

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ments such as urban air, petroleum distillates, coal tar, automobile exhaust, tobacco smoke, and marine and lake sediments. All these sources in the environment are likely contributors to the air pollution.

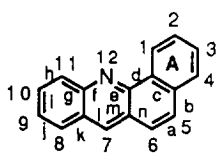
Benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) have not shown carcinogenic activity on mouse skin, whereas their 7-methyl derivatives show strong carcinogenic activity and many of their other derivatives are carcinogenic as well. The relatively large sizes of alkylated fused-ringed compounds found in some types of air pollution could cause a significant carcinogenic activity in the etiology of lung cancer [3–5]. Organic air pollutants can be subdivided into two regions: urban atmospheres and air pollution source effluents [6]. The quantum-chemical relationship between the structures of benzacridines and their carcinogenicity has recently been discussed [7–12], and the relative toxicological hazards of benzacridines vs. polycyclic aromatic hydrocarbons have been addressed.

Table 1
Molecular formula and molecular mass (*m/z*) of benzacridines

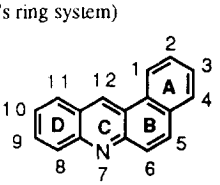
Compound	Mol. formula	Mol. mass (*m/z*)
Benzadridines		
Benzadridine	$C_{17}H_{11}N$	229
Monomethylbenzacridine	$C_{18}H_{13}N$	243
Dimethylbenzacridine	$C_{19}H_{15}N$	257
Trimethyllbenzacridine	$C_{20}H_{17}N$	271
Dibenzacridines		
Dibenzacridine	$C_{21}H_{13}N$	279
Monomethyldibenzacridine	$C_{22}H_{15}N$	293

Because of the apparent connection between benzacridines and cancer, chromatographic separation of the isomers of benzacridines was investigated [13–18]. Some of the investigated methodologies are reported below (Fig. 1) (Table 1).

1. Benzacridines (Four azaarene's ring system)

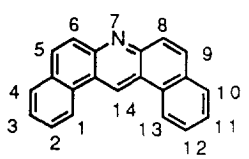


benz[*c*]acridine (**1**)
(1,2-benzacridine)

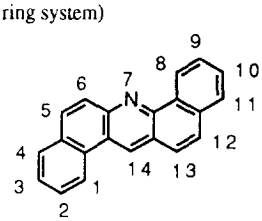


benz[*a*]acridine (**2**)
(3,4-benzacridine)

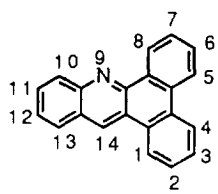
2. Dibenzacridines (Five azaarene's ring system)



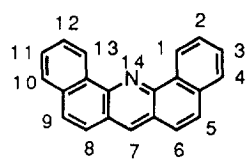
dibenz[*a,j*]acridine (**3**)
(1,2,7,8-dibenzacridine;
3,4,6,7-dinaphthacridine;
β-naphthacridine)



dibenz[*a,h*]acridine (**4**)
(1,2,5,6-dibenzacridine;
α-N-β-dinaphth-
acridine)



dibenz[*a,c*]acridine **(5)**
(1,2,3,4-dibenzacridine;
phenophenanthracridine)



dibenz[*c,h*]acridine **(6)**
(3,4,5,6-dibenzacridine;
α-naphthacridine;
α-N-α-dinaphthacridine)

Fig. 1. Benzacridines (four azaarene's ring system) and dibenzacridines (five azaarene's ring system).

2. Paper chromatography

Back to some decades, a dust extract was analyzed by paper chromatography using aqueous solvents [19,20].

3. Column chromatography

Benz[*c*]acridine (**1**), a common contaminant in automotive exhaust, has been found at concentrations of 200 μg/1000 m^3 gas by column chromatography on alumina. This was done by extracting the benz[*c*]acridine with 100 ml of pentane containing increasing amounts of diethyl ether, in increments of 8%, up to and including 64% [21].

The basic fractions in air pollution source effluents were column chromatographed on alumina with 100 ml of solvent mixtures at elution concentrations from 8% to 56% diethyl ether in pentane, 5% to 30% acetone in pentane, 100% acetone, and 100% methanol. Consequently, benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,j*]acridine (**3**) and dibenz[*a,h*]acridine (**4**) were determined at concentrations of 60, 18, 1.8 and 0.7 mg/1000 m^3 gas, respectively, via ultraviolet–visible spectrometry [22].

Collected particulates from urban atmospheres polluted by coal-tar-pitch fumes, from the effluent gases emanating from incinerators, and stacks of residential coal-burning furnaces were Soxhlet-extracted with benzene. The basic fraction was chromatographed on an aluminium oxide G column with 100 ml volumes of pentane solutions containing 8, 16, 24, 32, 40, 48 and 56% diethyl ether or 5, 10, 15, 20, 25, 30, 35 and 40% acetone followed by 100 ml volumes of 100% diethyl ether and then 100% methanol. Finally, each fraction of the eluents was separated on thin-layer (250 μm) plates coated with MN-cellulose powder 300 G using dimethylformamide–water (35:65) or with aluminum oxide G using pentane–diethyl ether (9:1). Air pollution source effluents such as a coal-tar-pitch fumes and coal-tar-pitch air samples analyzed by these procedures found benz[*c*]acridine (**1**) and its alkylated derivatives, benz[*a*]acridine (**2**) and its alkylated derivatives, dibenz[*a,j*]acridine (**3**), and dibenz[*a,h*]acridine (**4**) [23].

In 1963, samples of urban airborne particulates from the USA were separated by alumina column chromatography using pentane–diethyl ether (1:1). The samples were followed on cellulose thin-layer chromatography with fluorophotometry using dimethylformamide–water (65:35). Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,j*]acridine (**3**) and dibenz[*a,h*]acridine (**4**) in these urban atmospheres were detected at concentrations of 0.6, 0.2, 0.08 and 0.04 μg/1000 m^3 air, respectively [24].

For the detection of air pollutants from high-boiling petroleum distillates, separation was performed on the non-reactive base fraction using Amberlyst 15 cation-exchange resin. Then the base fractions were dissolved in cyclohexane and were separated by column chromatography with Cellex-P cation-exchange cellulose followed by chromatography on an acidic alumina column. After this eluate was treated, each eluted fraction was separated on a basic alumina column using cyclohexane–methylene chloride (9:1), methylene chloride, or absolute ethanol. Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and dibenzacridines in a subfraction were identified by mass spectrometry, fluorescence, and infrared spectrometry [25].

Asphaltenes and coal hydrogenation oils, obtained from Millmerran coal of Australia and New Wakefield of South Africa were separated by established procedures. The basic fraction was separated by Amberlyst 15 cation-exchange resin and fractionated by column chromatography on acidic and then basic alumina. Benz[*a*]acridine (**2**), from hydrogenation oil of New Wakefield coal and benz[*c*]acridine (**1**), dibenz[*a,h*]acridine (**4**), and dibenz[*a,j*]acridine (**3**), in the basic asphaltene fraction from a flash pyrolysis tar of Millmerran coal, were identified by comparison of the fluorescence emission and excitation spectra in both neutral and acid medium with library UV spectra [26]. Benz[*b*]acridine from Australian coal was identified by comparison of the fluorescence excitation spectra

in both neutral and acid medium with Library UV spectra [26]. All of these compounds {benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,j*]acridine (**3**) and dibenz[*a,h*]acridine (**4**) except benz[*b*]acridine} are known weak carcinogens [27].

4. Thin-layer chromatography

By using thin-layer chromatography with spectrophotofluorimetry, benz[*c*]acridine (**1**) and its carcinogenic alkylated derivatives, benz[*a*]acridine (**2**) and its carcinogenic alkylated derivatives, carcinogenic dibenz[*a,j*]acridine (**3**), and carcinogenic dibenz[*a,h*]acridine (**4**) have been found in air pollution source effluents [28].

Dibenz[*a,h*]acridine (**4**) was separated from an air sample polluted with coal-tar pitch by cellulose thin-layer chromatography with dimethylformamide–water (35:65) on an alumina column [29].

For the coal-tar pitch basic fraction, benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and dibenz[*a,h*]acridine (**4**) were found by two-dimensional thin-layer chromatography using two solvent systems such as cyclohexane–ethyl acetate (19:1) and dimethylformamide–water (35:65) on alumina–cellulose (2:1). Benz[*c*]acridine (**1**) was also detected by using two-dimensional thin-layer chromatography using solvent mixtures such as pentane and dimethylformamide–water (35:65) on alumina–cellulose (2:1), benz[*c*]acridine (**1**) was also detected from the basic benzene-soluble fraction of urban airborne particulates [30].

Benz[*c*]acridine (**1**) was also detected in a sample of air pollution source effluent through a Soxhlet extraction procedure with benzene–dimethylamine (4:1). The extracted residue was separated on a one-dimensional thin-layer alumina plate using pentane–diethyl ether (19:1) or on a two-dimensional thin-layer alumina plate using pentane–diethyl ether (19:1) and dimethylformamide–water (35:65), followed either by direct spectrophotofluorimetric analysis, elution and fluorimetric analysis, or by elution and filter spectrophotofluorimetric analysis [31].

Benz[*c*]acridine (**1**) also has been reported in urban atmospheres by Sawicki et al. [32].

These samples of urban and air pollution source effluents were Soxhlet-extracted with benzene–diethylamine (4:1). The treated residue was dissolved in methylene chloride and then developed on an alumina thin-layer plate using pentane–diethyl ether (19:1). Each spot adjacent to the standard samples was scraped off the plate. After treatment, the residue was dissolved in pentane–trifluoroacetic acid (50:1) and detected at $\lambda_{ex.}$ 282 nm/$\lambda_{em.}$ 475 nm by spectrophotofluorimetry. Benz[*c*]acridine (**1**) was found in the benzene-soluble fraction of urban airborne particulates and the detection limit of benz[*c*]acridine (**1**) in this study was shown to be 40 ng/ml [33].

Composite residues of benzene extracts of airborne particulates collected for 6 months in 1966 were dissolved in dichloromethane. The dichloromethane solution was developed by thin-layer chromatography on aluminum oxide G and silica gel G (1:1) using pentane–diethyl ether (19:1). Each spot was observed under a 360 nm light source, and the fluorescence areas of the standards and corresponding areas of the benzene-soluble sample were marked. Benz[*c*]acridine (**1**) in the sample area and the benz[*c*]acridine (**1**) standard were measured at $\lambda_{ex.}$ 290 nm/$\lambda_{em.}$ 470 nm by spectrophotofluorimetry. For example, benz[*c*]acridine (**1**) from the benzene-soluble fraction of suspended particulates collected in Los Angeles, Kansas City and New York from January to June 1966 were measured at concentrations of 0.2, 0.2 and 0.3 μg/1000 m^3 air, respectively. The city with the highest concentrations of benz[*c*]acridine (**1**) among 51 cities under investigation in the USA was Indianapolis, which had concentration levels of 1.5 μg/1000 m^3 air. Phoenix showed no detectable levels of this type of carcinogen. The concentrations of benz[*c*]acridine (**1**) in the 51 cities ranged from none detected to 1.5 μg/1000 m^3 air [34].

A tar fraction was collected from the air of a roadway tunnel and was Soxhlet-extracted with benzene–ethanol (3:1) and dissolved in benzene. The benzene solution was separated by two-

dimensional dual-band thin-layer chromatography coated with alumina G–Kieselguhr G (2:1) and 26% acetylated cellulose. Dibenz[*a*,*h*]acridine (**4**), 7,9-dimethylbenz[*c*]acridine and 7,10-dimethylbenz[*c*]acridine from gasoline powered vehicles were found at concentrations of 6.5, $1.8 \cdot 10^{-1}$, 1.2 μg/h, and $5.4 \cdot 10^{-1}$, 2.1, 6.0 μg/h, respectively. The average emission rate (μg/h) per vehicle of three benz[*c*]acridines from diesel vehicles was found to be higher than from gasoline engine vehicles. This was especially true of the average emission rates of 7,9-dimethylbenz[*c*]acridine and 7,10-dimethylbenz[*c*]acridine [35].

Pollution of groundwater and river water was investigated in Beijing, China. After partition separation of 12, 46, 70, and 10–70 m deep groundwater and river water, each basic fraction was separated by silica gel G thin-layer chromatography using *n*-pentane–ethyl acetate (19:1) and identified by mass spectrometry. Benz[*c*]acridine (**1**) was quantitatively determined by fluorometry at 384 nm. The highest concentration of benz[*c*]acridine (**1**) at 0.36 μg/l was found in 70 m deep groundwater. Other depths of groundwater and river water showed concentrations ranging from 0.01 to 0.193 μg/l. Benz[*a*]acridine (**2**) in the waters was determined qualitatively [36].

5. High-performance liquid chromatography

Azaarenes present in urban atmospheres have been analyzed by high-performance liquid chromatography (HPLC) coupled with on-line fluorescence detection after a pre-separation of the azaarene fraction by one-dimensional dual-band thin-layer chromatography. The HPLC column was a Zorbax ODS coupled with an Unisil ODS guard column with acetonitrile–water (7:3) as the mobile phase. The HPLC fraction was identified by comparison with retention times and fluorescence emission spectra of authentic azaarenes. Benzacridines from atmospheric particulate matter were analyzed in Tokyo in April 1983. Benz[*a*]acridine (**2**), dibenz[*a*,*j*]acridine (**3**), dibenz[*a*,*h*]acridine (**4**), and dibenz[*a*,*c*]acridine (**5**) were found at concentrations of 3.3, $4.3 \cdot 10^{-1}$, $3.6 \cdot 10^{-1}$, and $2.9 \cdot 10^{-1}$ μg/g of particulates, respectively [37].

A fast direct high-performance liquid chromatography (HPLC) method without pre-separation for determining azaarenes in atmospheric aerosols of Paris, France was achieved with high sensitivity. First, the samples were Soxhlet-extracted by dichloromethane–cyclohexane. The extract was separated by HPLC and fluorimetrically detected at variable wavelengths ($\lambda_{em.}$ 313–375 nm, $\lambda_{ex.}$ 366–425 nm). Picogram detection levels were obtained in the most favorable case. The HPLC column was a 5-m Vydac glass, 5 μm 201TP silica gel column using gradient solvents of 65–70% methanol with water and 70–100% methanol. Seven benzacridines have been determined at concentrations ranging from 0.49 ng/m^3 to 6.10 ng/m^3 in atmospheric aerosols from diesel motors and from 0.01 ng/m^3 to 0.19 ng/m^3 in atmospheric aerosol from gasoline motors, respectively. In this case, the detection limits of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) were both 4 pg/unit [38] (Table 2).

Azaarenes have been Soxhlet-extracted from cigarette smoke condensates on filter tips using chlorobenzene. The extract was pre-separated by liquid–liquid partition with a cation-exchange SP-Sephadex C25 glass column. Finally, the fractions were separated by HPLC with Cosmosil 5C18-AR or a reversed-phase ODS column with acetonitrile–water (3:1) as the mobile phase. Benz[*c*]acridine (**1**) and 9-methylbenz[*c*]acridine in cigarette filter tips were measured at concentrations of 0.37 ng/tip and 0.11 ng/tip, respectively. The detection limit was determined to be 5 pg/tip [39].

Creosote oils were treated with liquid–liquid partition. The basic portion was transferred to a Sephadex LH 20 column and followed by a SP-Sephadex C25 cation-exchange column. The pre-separated basic fraction was separated by HPLC with a pre-packed Ultron S C_{18} column with acetonitrile–water (7:3) as the mobile phase. The sub-fractions were again separated by HPLC with a TSK gel 120T reversed-phase column and followed by thin-layer chromatography (HPTLC) on a pre-coated RP-18 column with an

Table 2
Benzacridines in an atmospheric aerosols

Compound	Source	
	Diesel (ng/m^3)	Petrol (ng/m^3)
Benzacridines		
Benz[*c*]acridine (**1**)	5.80	0.07
Benz[*a*]acridine (**2**)	6.10 ± 0.5	0.19
2-Methylbenz[*a*]acridine	1.12	0.01
7-Methylbenz[*a*]acridine	0.49	0.01
Dibenzacridines		
Dibenz[*a,j*]acridine (**3**)	0.84 ± 0.8	
Dibenz[*a,h*]acridine (**4**)	0.82	
Dibenz[*c,h*]acridine (**6**)	0.72	

acetonitrile–chloroform (4:1) mobile phase. The basic fraction was scraped from TLC plate and measured by fluorescence spectrometry. The basic sample was analyzed by gas chromatography on a fused-silica capillary column with bound OV-1 equipped with a thermionic specific detector (TSD). The identification of azaarenes in the creosote oils was achieved by gas chromatography–mass spectrometry (GC–MS) connected with a mass selective detector. Benz[*c*]acridine (**1**), 9-methylbenz[*c*]acridine, 10-methylbenz[*c*]acridine in creosote oils have been detected at concentrations of 192.7, 7.7 and 18.4 μg/g, respectively [40].

6. Gas–liquid chromatography

Sawicki found benz[*c*]acridine (**1**) and dibenz[*a,h*]acridine (**4**) as a composite average in American urban air in 1963 at 1 μg/1000 m^3 and $2 \cdot 10^{-1}$ μg/1000 m^3, respectively [41]. Four-fused ring benzacridines, benz[*c*]acridine (**1**) and its related carcinogenic methylated benz[*c*]acridines, and benz[*a*]acridine (**2**) and its related carcinogenic methylated benz[*a*]acridines were all found in urban American atmospheres [3,41]. Additionally, five-fused ring benzacridines, carcinogenic dibenz[*a,h*]acridine (**4**), and dibenz[*a,j*]acridine (**3**) with its related alkylated carcinogenic dibenz[*a,j*]acridines have been found in the same cities [3,41].

Dust samples were collected from the air by means of an high-volume Staplex pump. The pump was situated about 15 meters above the ground. Samples of 0.3–0.5 g of dust collected from 1000 to 2000 m^3 air were Soxhlet-extracted with cyclohexane. After extraction and chemical treatments, the basic samples were analyzed by GC with a glass capillary column coated with SE-52 at 180°C, with flame ionization or electron-capture detection. Consequently, benz[*c*]acridine (**1**), 10-methylbenz[*c*]acridine, 10-methylbenz[*a*]acridine, 1,10-dimethylbenz[*a*]acridine and 8,10-dimethylbenz[*c*]acridine were found in these atmospheric dust extracts [20].

Tobacco was pyrolyzed at 850°C in an atmosphere of nitrogen. The various fractions were then analyzed by GC on a stainless-steel column containing 15% Carbowax on Chromosorb W, equipped with a flame ionization detector. Thin-layer chromatography on each basic fraction extract was carried out on silica gel G using ethyl acetate–methyl alcohol–formic acid (80:10:10) and benzene–methyl alcohol (95:5). Benzacridines have been found in tobacco of the pyrolyzates and tobacco smoke condensate. Dibenzacridines could not be found in this study [42]. However, Van Duuren et al. reported that dibenzacridines were found in tobacco smoke, in

nicotine and pyridine pyrolysates [43]. Dibenzacridines found also in nicotine itself.

Airborne particulate matter was analyzed in buildings in a residential area of Antwerp, Belgium. Samples were collected on Whatman GFA glass fiber filters. After pretreatment, a benzene-soluble basic fraction was separated by GC on a 5-m packed column containing 4% Dexsil 300 on Gas Chrom Q 100–120 mesh support. The determinations were carried out with a Finnigan Model 3100 gas chromatograph–mass spectrometer. These samples showed azabenz[*a*]anthracenes containing four isomers of molecular mass 229 {benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**)}, four isomers of molecular mass 243 (including methylbenzacridines), and two isomers of molecular mass 279 {including dibenz[*a*,*h*]acridine (**2**) and dibenz[*a*,*j*]acridine (**3**)}. These were detected at concentrations of 16, 3, and 4 ppm, respectively [44].

A raw shale oil was extracted and separated into five complex fractions. These were analyzed by GC–MS. The raw shale oil and a basic fraction were found to be mutagenic against the *Salmonella typhimurium* test strains such as TA98 and TA100. Mutagenicity was dependent on metabolic activation by microsomal (S9) enzyme reactions which form active mutagens from premutagenic precursors such as metabolic activation reactions. In this regard, both inhibition and inactivation of S9 enzymes are possibilities that must be considered. The effect of a neutral arene's fraction on the mutagenicity of 7,9-dimethylbenz[*c*]acridine was determined by the Ames assay. Premutagen, 7,9-dimethylbenz-[*c*]acridine, requiring metabolic activation was added to the basic and neutral arene fractions, and the numbers of revertants obtained in the presence of the fractions were compared with mutation induced by 7,9-dimethylbenz[*c*]acridine alone. The mutagenicity of 25 μg/plate of 7,9-dimethylbenz[*c*]acridine as a function of increasing arene concentration was inhibited in much the same way as observed for benzo[*a*]pyrene and dibenzanthracene. On the other hand, the response curve observed for the concentrations from 10 to 50 μg/plate of 7,9-dimethylbenz[*c*]acridine was similar in the levels of maximum response in the presence or absence of the basic fraction. However, the rates of mutagenesis were somewhat reduced by the basic fractions. This fact suggests that a fraction from the raw shale oil could also contain dimethylbenz[*c*]acridines in small amounts [45].

Additionally, the relationship between a basic fraction and carcinogenicity by the Ames test has been shown by Pelroy et al. [46]. The raw shale oil was fractionated into five sub-fractions. Both basic and arene subfractions were most mutagenic in *S. typhimurium* strains TA98 and TA100. 7,9-Dimethylbenz[*c*]acridine showed more of the mutagenicity in the presence of shale oil than did benzo[*a*]pyrene. The basic shale oil fraction was also toxic to *S. typhimurium* sTA100 over the same concentration range required for mutagenesis and this toxicity was enhanced by metabolic activation [46].

A sub-fraction of ether-soluble bases from petroleum crude oils and coal- and shale-derived petroleum substitutes was separated first on a basic alumina column using benzene. The extracted sample was eluted first with benzene and then ethanol. After the ethanol was removed, the residue was dissolved in 2-propanol then transferred to a Sephadex LH-20 column first using 2-propanol and then acetone as the mobile phase. Each elution sample was separated by GC on 3% Dexsil 400 and detected with MS. Benzacridine was found in an acetone sub-fraction of the petroleum substitutes [47].

The extract from coal liquefaction products have been analyzed for benzacridine molecules. Coupled GC [with flame ionization detection (FID) and nitrogen-selective alkali-flame detection (AFD)–mass spectrometry (GC–MS)] before and after derivatization with dimethylformamide dimethylacetal (Methyl-8) was utilized. Splitless injections of 0.2 μl were made onto a 40-m SGE glass support-coated open tubular (SCOT) capillary column which was coated with SP-2250 (methyl silicone–phenyl silicone, 1:1) stationary phase. Hydrogen carrier gas was used for FID analyses and helium for AFD analyses. The basic fraction sample was analyzed by GC–FID using dibenzyl as an internal standard. From these studies, benz[*a*]acridine (**2**) with a molecu-

lar mass 229 was found at a concentration of 10 ppm in a basic fraction of the coal product [48].

Groundwater samples obtained from a well are a mixture of oily-tar and aqueous phases. A fluid sample was obtained from well W13 located 205 m south of the site of the former coal-tar distillation plant in St. Louis Park, MN, USA. A composite sample was allowed to stand undisturbed in a separatory funnel so that the two phases were gravity separated. The lower black, oily-tar phase with the higher density was separated from the brown, aqueous phase by centrifugation at 4000 rpm in a high-speed centrifuge and filtered under vacuum through a glass fiber filter (Gelman, Type A–E). Organic bases were isolated from each phase by pH adjustment and solvent extraction. Organic bases in the oily-tar phase were further purified by micro-column adsorption chromatography with neutral alumina. The separation and identification of the organic bases in each phase were achieved by automated capillary GC–MS and probe distillation–high resolution mass spectrometry (PD–HRMS) techniques. The gas chromatograph was equipped with a wall-coated open tubular, fused-silica capillary column coated with SE-54. By a reconstructed ion chromatogram (RIC) of the base fraction of the oily-tar phase from the groundwater, two isomers of benzacridine and two isomers of dibenzacridine were detected. Further analysis of the organic bases in the oily-tar phase of groundwater by automated GC–MS identified benzacridine with molecular mass 229, its monomethylated derivative with molecular mass 243, its dimethylated derivative with molecular mass 257, and three isomers of dibenzacridine with molecular mass 279 and one monomethylated derivative with molecular mass 293 [49].

The polycyclic aromatic compound fraction from diesel fuels was isolated on a silica gel column. Filter paper exposed to diesel exhaust isolated the polycyclic aromatic compound fraction which was then Soxhlet-extracted using benzene–methanol. After appropriate dilution, the samples of the polycyclic aromatic compound fraction were spiked internally with standards for quantification purposes. The samples were analysed on a gas chromatograph, which employed a novel three-way effluent splitter thus enabling simultaneous parallel detection of the standards by means of FID, and nitrogen selective (NPD), and sulphur selective (SSD) detectors. The instrument was equipped with a cold on-column injector and a silica capillary column, coated with cross-linked SE-54. Benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) were determined at concentrations lower than 1 ppm in both diesel fuels and gas oils [50].

Samples from an anthracene oil, a coke oven pitch and a low-temperature coal tar were obtained as unfractionated materials. The bases were separated on SGE glass support-coated open tubular (SCOT) capillary columns coated with an SP-2250 (50% methylphenyl silicone–50% phenyl silicone) stationary phase, dual gas chromatography–alkali flame detection (AFD) and gas chromatography–flame ionization detection (FID). Consequently, benz[*a*]acridine (**2**) in anthracene oil, coke-oven pitch and Gray-King tar in coal tar products was detected by GC–AFD at concentrations of 400, 730 and 84 ppm, respectively. Additionally, identification was determined by drawing single-ion chromatograms for normal m/z values corresponding to possible azaarenes [51].

Basic effluents of anthracene oil of coal tar products were separated by aqueous acid extraction of dichloromethane, cation-exchange chromatography on Amberlyst 15, liquid chromatography on a polar, bonded-phase silica OPN/Porasil C Durapak and organometallic coordination chromatography on anhydrous $FeCl_3$/Chromosorb W. The basic fraction was analyzed by GC–FID. Splitless injections of 0.2 μl were made onto a 40-m SGE glass support-coated open tubular (SCOT) capillary column coated with an SP-2250 (50% methylsilicone–50% phenylsilicone) stationary phase, using hydrogen as the carrier gas. For the identification of azaarenes in the basic fractions, GC–MS was used. The chromatograph was interfaced with a Kratos MS-30 double-beam mass spectrometer/DS-55 data system. Splitless injections of 1–5 μl were made on a 33-m SGE SP-2250 glass SCOT capillary column under chromatographic condi-

tions similar to those used for the GC–FID analyses. Benz[*a*]acridine (**2**) with *m/z* 229 at concentrations of 363, 349, 418 (a neutral nitrogen fraction separated by hexane–15% benzene), and 473 ppm in basic fractions from an anthracene oil was detected by aqueous acid extraction, cation-exchange chromatography, liquid chromatography on OPN/Porasil C Durapak and co-ordination chromatography on $FeCl_3$/Chromosorb W, respectively [52].

Anthracene oils were separated by liquid chromatography on a Waters 80–100 mesh OPN/Porasil Durapak, with a glass Whatman 'MultiSystem' chromatographic column. The fraction samples were analyzed by GC using a Perkin-Elmer F-17 chromatograph fitted with a flame-ionization detector and a nitrogen-selective alkali flame detector. Splitless injections of 0.2 μl were made onto a 40-m SGE glass SCOT capillary column coated with SP-2250 (50% methyl silicone–50% phenyl silicone) stationary phase. The neutral fraction was analysed by GC–FID and benz[*a*]acridine (**2**) in the neutral nitrogen fraction (hexane–15% benzene eluate) was at a concentration of 418 ppm [53].

A sample of a solvent refined coal material (SRC II) was obtained from the processing of Pawhatan Mine No. 5 coal on Process Development Unit P-99 operated by Gulf Science and Technology Co. at Harmarville, PA, USA. The material was fractionally distilled to produce the following five boiling point cuts: 300–700, 700–750, 750–800, 800–850 and 850°F + bottoms. The five distillate cuts were fractionated by an adsorption alumina column chromatography to obtain arene and azaarene fractions. Then, the azaarene fraction was identified by a fused-silica DB-5 capillary column with GC–FID detection, in conjunction with a HP5982A capillary gas chromatograph mass spectrometer. Benz[*c*]acridine (**1**) of the three SRC II boiling point cuts 700–750, 750–800 and 800–850°F has been found at concentrations of 578, 1237 and 2357 ppm, respectively [54].

Several integrated two-stage coal liquefaction (ITSL) samples were collected from ITSL process plant at C.E. Lummus in New Brunswick, NJ, USA. The samples were fractionated by alumina column chromatography. Further chromatographic separation isolated azaarene fractions. High-resolution gas chromatography using a 25-m fused-silica capillary column coated with DB-5 and FID, coupled with a mass spectrometer, was used to determine the concentrations of the azaarene fractions of the ITSL. Concentrations in parts per thousand (ppt) of three benzacridine fractions, benz[*c*]acridine (**1**), 7,10-dimethylbenz[*c*]acridine and dibenz[*a,j*]acridine (**3**) (or dibenzo[*a,j*]carbazole or an isomer), of 32 azaarenes measured in azaarene fractions of ITSL materials were found at concentrations ranging from 1.3 to 2.6, 0.10 to 5.8 and 0.04 to 0.63 ppt, respectively [55].

Organic pollutants from airborne particulates in the Taiyuan area of China were analyzed on a 25-m flexible quartz capillary Dexsil-300 column-gas chromatograph. Benz[*c*]acridine (**1**) was determined at concentration of 33.51 ng/m^3 and showed carcinogenicity in the Ames test [56].

A river sediment sample was Soxhlet-extracted by using dichloromethane and then the extract was pre-separated onto a Sephadex LH-20 column using 2-propanol. The azaarene fraction was analysed on a fused-silica capillary OV-101 glass column with GC–FID and a coupled mass spectrometer. Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**4**) and dibenz[*a,c*]acridine (**5**) were qualitatively detected in these sediment samples [57].

Sediments collected in Eagle Harbor, Puget Sound, WA, USA and commercial creosotes were analyzed for azaarenes by capillary GC with NPD, FID, and MS. The organic sediment extracts and the creosotes were fractionated by silica gel/alumina column with methanol–methylene chloride (1:4) as the mobile phase. The basic fractions were separated on a gas chromatograph with a fused-silica gel capillary column bonded with SE-54, equipped with a thermionic-specific (nitrogen-phosphorus) detector. The bonded-phase SE-54 fused-silica gel capillary column was interfaced to a Finnigan 3200 MS. Concentrations were confirmed by comparison with spectra obtained from the standard samples analyzed under the identical conditions. Benzacridine and all isomers of molecu-

lar mass 229, and methylbenzacridine and all isomers of molecular mass 243 in the sediment were measured at concentrations ranging from 7.7 to 8.5 μg/g, and from 1.0 to 1.4 μg/g, respectively. Benzacridine and all isomers of molecular mass 229, and methylbenzacridine and all isomers of molecular mass 243 in creosote were measured at concentrations ranging from 600 to 4900 μg/g and from 71 to 110 μg/g, respectively [58].

Azaarenes from samples of airborne particulate matter in Copenhagen collected during 5 years were extracted by toluene with an ultrasonic treatment. Isolated basic azaarenes were extracted twice with 8.25 *M* phosphoric acid. Both phosphoric acid phases were combined and adjusted to about pH 14 with 11 *M* potassium hydroxide. The azaarenes were extracted with dichloromethane and determined by GC with a fused-silica column Ultra-1 equipped with a nitrogen-sensitive detector (NPD). Air samples were collected in February from 1976 to 1982 in suburban, residential areas and busy streets of Copenhagen. Benz[*a*]acridine (**2**), dibenz[*a*,*j*]acridine (**3**) and dibenz[*a*,*h*]acridine (**4**) were detected at 0.09, 0.2, 0.2 ng/m^3 in the residential area and 0.17, 0.07, 0.08 ng/m^3 in the busy street area, respectively [59].

Particles and semi-volatiles in main- and sidestream smoke of cigarettes were collected. The separation of basic fractions was achieved by a S-Sepharose ion exchange chromatography, Sephadex LH20, and then again an S-Sepharose. The fraction was then separated by gas chromatography on a fused-silica capillary column coated with SE-54 and interfaced with a nitrogen-flame ionization detector (N-FID). When the chromatograms were compared with the standard samples, benz[*c*]acridine (**1**) in a sidestream smoke was found [60].

Air pollution particulates collected in Calcutta, India, were consecutively extracted with benzene and methanol. The samples were separated by gas chromatography on a fused-silica 25-m capillary column coated with Sil 5 and coupled with a mass spectrometer. Benz[*c*]acridine (**1**), and an isomer mixture of dibenz[*a*,*j*]acridine (**3**) and dibenz[*a*,*h*]acridine (**4**) were at concentrations ranging from 1.06 ng/m^3 to 4.76 ng/m^3 and from 7.27 ng/m^3 to 13.16 ng/m^3, respectively. The concentrations of three azaarenes found in Calcutta during January–February 1984 were higher than the results from Wilrijk, Belgium [61].

7. Conclusions

Different techniques are available and have been used for the chromatographic determination of benzacridines. The methods discussed in this review include paper chromatography, column chromatography, thin-layer chromatography, high-performance liquid chromatography and gas–liquid chromatography.

Paper chromatography, although used some decades ago, is no longer one of the major techniques used for quantitative determination of benzacridines. In the case of column chromatography, alumina (adsorption chromatography) and ion exchange resins were employed.

Thin-layer chromatography (on alumina, alumina–cellulose, alumina–silica gel, etc.) is a very convenient and rapid technique used in the determination of benzacridines in polluted air and in ground and river water.

High-performance liquid chromatography has been successfully used in Tokyo and Paris for analysis of urban atmospheres as well as in other situations.

Finally, gas–liquid chromatography, as a convenient, and highly successful method, has been widely used as the principal method of analysis in numerous publications devoted to air pollution (urban atmospheres), tobacco, cigarette smoke, shale oils, petroleum, diesel fuel, coal liquefaction products, coal tar, groundwater, etc.

All these methods are being constantly improved upon, with various modifications leading to a higher accuracy and reliability of results.

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