Journal of Chromatography, 302 (1984) 43–50
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 408

TRACE ANALYSIS OF ORGANIC COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION

J. N. DRISCOLL*, D. W. CONRON and P. FERIOLI
Research and Development Department, HNU Systems, Inc., 160 Charlemont Street, Newton Highlands, MA 02161 (U.S.A.)
and
I. S. KRULL and K.-H. XIE
Barnett Institute and Department of Chemistry, Northeastern University, 360 Huntington Avenue, Boston, MA 02115 (U.S.A.)

SUMMARY

Photoionization detection (PID) has now been successfully interfaced with modern high-performance liquid chromatography (HPLC) to provide a new "hyphenated" technique, HPLC–PID. This method is compatible with reversed-phase HPLC solvents and conditions, as well as with certain organic solvents used in normal-phase liquid chromatography. The HPLC–PID interface consists of a variable-ratio eluent splitter, operated inside a heated oven, which vaporizes both analytes and mobile phase prior to introduction into the detector. Depending on the energy (eV) of the photoionization detector lamp, most of the solvents commonly used in reversed-phase liquid chromatography are compatible with long-term, on-line, real-time, continuous photoionization detector operation. Virtually all organic classes now detectable by gas chromatography–PID can also be detected by HPLC–PID, but with somewhat poorer detection limits. In certain instances, minimum detection limits are in the 5–500 ng/injection range. The HPLC–PID system appears ideal for aromatic or aliphatic amines, substituted hydrocarbons, and certain other classes of organic compounds. Various applications of HPLC–PID, under reversed-phase conditions are illustrated and discussed.

INTRODUCTION

Photoionization detection (PID) has been employed in gas chromatography (GC) for nearly a decade, providing the "hyphenated" technique GC–PID[1–8]. Enhanced selectivity and improved sensitivity have been demonstrated over the years for many classes of compounds, especially when multiple detection is employed, *e.g.*, electron-capture detection (ECD)–PID, PID–PID, etc. Although GC–PID and other GC–PID–detector combinations have been described in the literature[1–8], very little has yet appeared in the area of high-performance liquid chromatography (HPLC)–

PID interfacing[9–11]. Locke and co-workers[10,11] and Driscoll and Becker[9] have reported some preliminary data on the interfacing of both normal- and reversed-phase HPLC with PID. However, no recent work has been described that would suggest that HPLC–PID is a viable, easy-to-use, highly specific method of organic trace analysis. In general, problems in HPLC–PID arise from a variety of sources, such as: (1) quenching of the photoexcited analyte by mobile-phase molecules before the analyte ions can be collected and detected, which leads to poorer detection limits than GC–PID for the same organic analytes; (2) requirement for vaporization of the sample and, hence, a stable liquid–gas interface.

In our initial work on HPLC–PID, we used a heated interface oven similar to that previously described for HPLC–ECD[12,13]. The heated interface provides for complete volatilization of the reversed-phase eluents, a variable ratio splitting of the vaporized eluents, and mixing of the vaporized eluents with a carrier gas, all prior to introduction into the photoionization detector. Use of a heated interface for HPLC–PID is fully compatible with aqueous and organic mobile phases, and it provides suitable detection limits for several classes of analytes. Detection limits may be improved somewhat, for particular analyte classes, by using a PID lamp other than the 10.2 eV model. Detection limits have also been shown to be affected by the nature of the carrier gas used in the interface oven.

HPLC–PID has now been evaluated, with regard to minimum detection limits (MDL), concentration ranges of linearity, analyte specificity, and application to specific classes of analytes with the use of reversed-phase HPLC solvents. These approaches suggest that PID can be a useful and practical selective detection method for HPLC, with a sensitivity dependent on the particular analyte compound or class.

EXPERIMENTAL

The HPLC apparatus (Fig. 1) consisted of the following items: (1) a Waters Model U6K injection valve (Waters Assoc., Milford, MA, U.S.A.) or a Rheodyne Model 7125 syringe-loading injection valve (Rheodyne Corp., Cotati, CA, U.S.A.); (2) a LDC Constametric III solvent delivery system (Laboratory Data Control Div., Milton Roy, Riviera Beach, FL, U.S.A.); (3) an Alltech C_8 or C_{18} reversed-phase 10 μm, 25 cm × 4.6 mm I.D., analytical column (Alltech, Deerfield, IL, U.S.A.), or a Waters μBondapak C_{18}, 10 μm, 25 cm × 4.6 mm I.D. column (Waters Assoc.); (4)

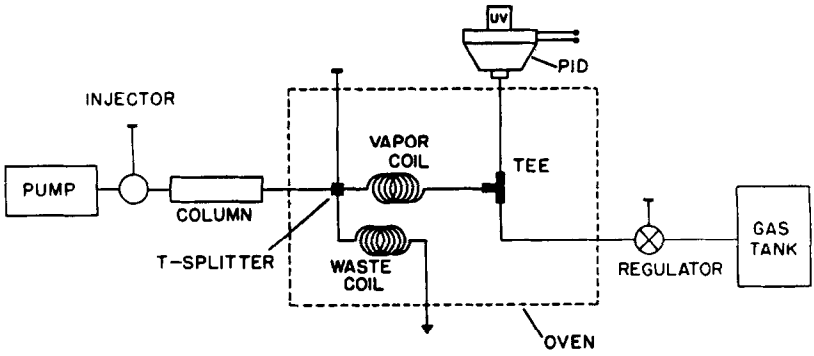


Fig. 1. Schematic diagram of the HPLC–PID apparatus in operation.

an LDC Model 1203 fixed-wavelength UV–VIS detector (254 nm); (5) a Linear Model 585 dual-pen recorder (Linear Instruments, Irvine, CA, U.S.A.); (6) an HNU Systems Model PI-52 photoionization detector and associated electrometer (HNU Systems, Newton Highlands, MA, U.S.A.); a variable-ratio splitting tee (Alltech); (8) an HNU Model 301 or 401 GC oven, which was used as the heated interface oven for the HPLC–PID system (Fig. 1).

The HPLC separations were performed on a C_8 or C_{18} analytical HPLC column, with mobile phases of acetonitrile–water or methanol–water at flow-rates from 0.5 ml/min to 2.5 ml/min. The detection limits (based on peak height) could be improved by a factor of two by reducing the solvent flow-rates from 2.5 to 0.5 ml/min. The peak area increased four times over the same range, since the photoionization detector is a concentration-sensitive detector. The HPLC–PID split ratio dividing the eluent between the waste and the fraction directed to the photoionization detector was operated at about 1:1. The carrier gas used was generally helium, at a flow-rate of 20–30 ml/min, before the split to the photoionization detector. The effect of carrier gas flow-rate on sensitivity was evaluated over the range from 5–60 ml/min. The region between 20–30 ml/min was chosen for the following reasons: problems with reproducibility at lower flows, reduced sensitivity at flow-rates > 40 ml/min, and reasonably small variations (*ca.* 10%) in sensitivity in the range of 30–40 ml/min. A number of PID lamps were evaluated, ranging in energy from 8.3 to 10.2 eV. The vaporization-interface oven was operated at about 240–250°C, and the photoionization detector temperature was maintained at about 290–300°C. Retention times were measured in duplicate or triplicate with a stop watch. Detection limits were determined as the minimum amount (mass) of compound necessary to produce a signal-to-noise ratio (peak height) of at least 2:1 at the maximum sensitivity attainable on the photoionization detector electrometer and associated recorder.

RESULTS AND DISCUSSION

We first attempted to optimize all the conditions including carrier gas flow, lamp energy, and mobile-phase flow-rate. Then we determined the sensitivity and detection limits for a number of organic solutes.

The PID responses as a function of the carrier gas used, for a limited number of analytes, have been determined, and the data are given in Table I. Helium appears to provide the greatest sensitivity and minimum detection limits for almost all of the compounds studied thus far. Thus, for all of the remaining studies and applications, helium has been the reagent gas of choice in HPLC–PID.

The effect of different PID lamp energies on the relative responses of various organic analytes has also been determined, using normalized responses (Table II). For some of these compounds (the ones with ionization potentials in the 7–8-eV range), the 9.5-eV lamp provides one order of magnitude improvement in the PID response, while for other analytes the ionization potentials are in the range 8.5–9.5 eV; these responses are decreased in comparison with the 10.2-eV lamp data.

We have applied the above optimized HPLC–PID apparatus and conditions to various classes of organic compounds, including polycyclic aromatic hydrocarbons (PAH) shown in Fig. 2, substituted anilines (Fig. 3), and aliphatic ketones (Fig. 4). The analytes were injected not at their MDL, but usually at levels of several μg per

TABLE I

RELATIVE PID RESPONSES TO SELECTED ANALYTES WITH DIFFERENT CARRIER GASES

Mobile phase, water–acetonitrile (50:50, v/v); flow-rate, 0.72 ml/min; no split ratio in use; 10.2-eV lamp; interface oven at 240°C; photoionization detector at 295°C. Normalized responses (nitrogen = 1.00) were measured in terms of cm or mm peak height per ng or μg of compound entering the photoionization detector.

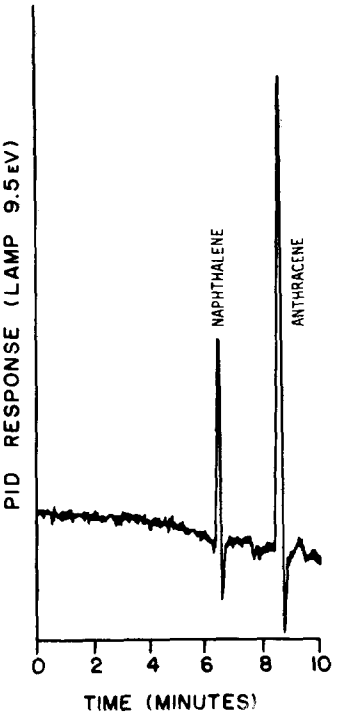
Carrier gas	*Normalized responses*							
	Naphthalene	*o-Nitro-toluene*	*3-Hexanone*	*2-Nitro-naphthalene*	*N,N'-Dimethyl aniline*	*N-Nonylamine*	*Triamylamine*	*N,N'-Dimethyl formamide*
Nitrogen	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Argon	2.00	2.67	2.08	2.59	3.42	2.60	2.93	2.90
Helium	2.67	3.03	2.16	3.11	3.33	3.28	2.84	3.39

TABLE II

RELATIVE PID RESPONSES TO ORGANIC COMPOUNDS AT DIFFERENT LAMP ENERGIES (eV)

Mobile phase, water–acetonitrile (50:50, v/v); flow-rate, 0.72 ml/min; carrier gas (helium) flow-rate, 20 ml/min; interface oven at 240°C; photoionization detector at 295°C; no HPLC column in line. Normalized photoionization detector responses were determined by measuring peak height (cm or mm) on a per ng or μg basis for amounts of analytes reaching the photoionization detector (10.2-eV response = 1.00). NR = no response observed for these compounds.

Lamp energy (eV)	*Normalized photoionization detector responses*							
	Naphthalene	*o-Nitro-toluene*	*3-Hexanone*	*2-Nitro-naphthalene*	*N,N'-Dimethyl aniline*	*N-Nonyl-amine*	*Triamyl amine*	*N,N'-Dimethyl formamide*
10.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9.5	5.55	0.014	0.024	0.14	11.0	0.21	9.69	0.019
8.3	0.08	NR	NR	NR	0.30	0.012	0.17	NR



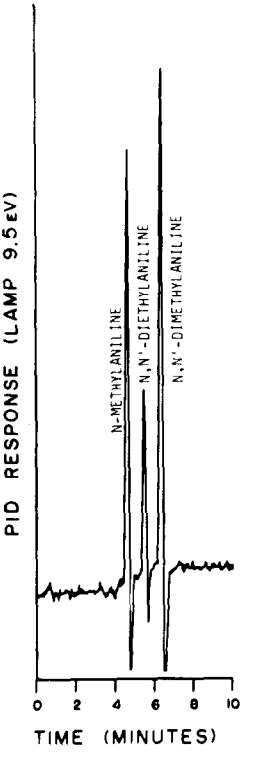
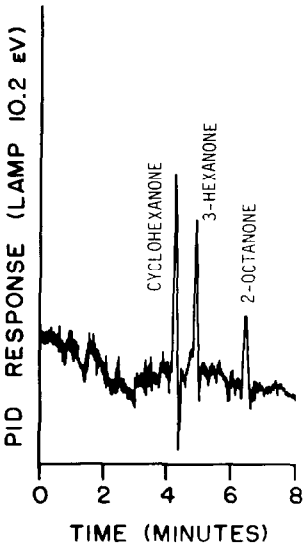


Fig. 2. The HPLC–PID chromatogram of naphthalene (6 μg) and anthracene (5.5 μg). HPLC column C_8, 10 μm, 25 cm × 4.6 mm I.D.; mobile phase, water–acetonitrile (25:75, v/v); flow-rate 0.8 ml/min, split ratio, 7:3 (PID–waste); 9.5-eV lamp; interface oven at 240°C; photoionization detector temperature, 290°C; attenuation setting, 2×10^{-10} a.u.f.s.

Fig. 3. The HPLC–PID chromatogram of three N-substituted anilines, N-methylaniline, N,N'-diethylaniline, and N,N'-dimethylaniline. Column, C_8, 10 μm, 25 cm × 4.6 mm I.D.; mobile phase, water–acetonitrile (25:75, v/v); flow-rate, 0.8 ml/min; split ratio, 7:3 (PID–waste), 9.5-eV lamp; interface oven at 240°C; photoionization detector temperature at 290°C; attenuation setting, 2×10^{-10} a.u.f.s.

injection. Fig. 2 is a HPLC–PID chromatogram for naphthalene and anthracene, both at the 5–6 μg/injection level (20 μl), obtained by using water–acetonitrile (25:75) as the mobile phase. Fig. 3 is the HPLC–PID chromatogram of three substituted anilines, again injected at the low μg/injection-levels. Fig. 4 is a HPLC–PID chromatogram of three aliphatic ketones, cyclohexanone, 3-hexanone, and 2-octanone, 10–16 μg/injection. One additional application of HPLC–PID (Fig. 5) was under normal-phase HPLC conditions with a mobile phase of isopropanol–hexane (3:97). We analysed a mixture of the same three substituted anilines as in Fig. 3. It is interesting to note that both hexane and isopropanol have ionization potentials low enough (about 10 eV) to be ionized by the 10.2-eV lamp. All of the reversed-phase solvents used have ionization potentials greater than 10.5 eV and would not be ionized. Clearly, these analyses can be used with both reversed- and normal-phase solvents. There does not appear to be a significant problem of band broadening or excessive variances when the heated interface-vaporization oven is placed between the HPLC apparatus and the photoionization detector. Even with the reversed-phase



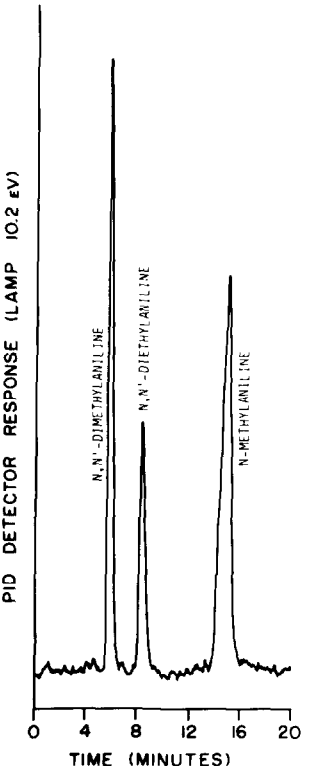


Fig. 4. The HPLC–PID chromatogram of three aliphatic ketones; cyclohexanone (16 μg), 3-hexanone (12 μg), and 2-octanone (10 μg). Column, C_8, 10 μm, 25 cm × 4.6 mm I.D.; mobile phase, water–acetonitrile (25:75, v/v); flow-rate, 0.8 ml/min; split ratio of 1:1 (PID–waste); 10.2-eV lamp; interface oven at 240°C; photoionization detector temperature at 290°C; attenuation setting, 4×10^{-12} a.u.f.s.

Fig. 5. The HPLC–PID chromatogram of N-substituted anilines on a LiChrosorb Si60, 5 μm, 25 cm × 4.6 mm I.D. column. Mobile phase: 3% isopropanol–hexane; flow-rate, 0.46 ml/min; split ratio of 3:7 (PID–waste); 10.2-eV lamp; interface oven at 230°C, photoionization detector temperature at 290°C; attenuation setting 1×10^{-12} a.u.f.s.

solvents, there is good baseline stability, and noise was not a serious problem at the levels we injected. Overall, chromatography gives acceptable results, peak shape is fairly good, baseline resolution is obtained in all cases, and the total analysis times are within reason.

The photoionization detector would therefore appear to be similar to other currently employed HPLC detectors, such as the UV–VIS, fluorescence, or electrochemical detectors. HPLC–PID appears to be most suitable for classes of compounds substituted with electron-absorbing groups. The order of response in Table III indicates that strongly electronegative groups on a molecule improve the MDL. Note, for example, the improvement in sensitivity going from benzene to xylene to phenol or halogenated benzenes. The actual mechanism is still uncertain, although it appears that the dipole, induced by the electronegative group, prevents quenching of the ionized species prior to collection. The electronegativity effect may also be responsible for the negative peak seen in Figs. 2–4. Table III summarizes the best MDL that are

TABLE III

MINIMUM DETECTION LIMITS FOR CERTAIN ORGANIC COMPOUNDS BY HPLC-PID

HPLC conditions: Alltech C_8 column, 10 μm, 25 cm × 4.6 mm I.D.; mobile phase, acetonitrile–water (75:25, v/v); flow-rate, 0.8 ml/min, directly to photoionization detector.

Organic compounds	*Minimum detection limit (ng)*	*Lamp energy (eV)*
Bromobenzene	3	10.2
Iodobenzene	4	10.2
Phenol	4	10.2
Chlorobenzene	5	10.2
N,N'-Dimethylaniline	5	9.5
Dioctylphthalate	5	10.2
Fluorobenzene	7	10.2
N,N'-Diethylaniline	20	9.5
N-Methylaniline	60	9.5
o-Xylene	60	10.2
Anthracene	75	9.5
Benzene	150	10.2
Naphthalene	200	9.5
2-Octanone	125	10.2
N-Methylformamide	500	10.2
N,N'-Dimethylformamide	500	10.2
N,N'-Diethylformamide	600	10.2
3-Hexanone	700	10.2

obtainable thus far with the approaches and instrumentation already described. Detection limits in GC–PID for all of the analytes indicated in Table III are several orders of magnitude lower than now attainable by HPLC–PID methods. One approach that might lead to an improved MDL for these and other classes of compounds may be the use of off-line or on-line derivatizations for certain classes of compounds, if such derivatives have improved overall sensitivities in HPLC–PID[14]. However, there is no guarantee that, even with such derivatives, the final MDL will indeed be several orders of magnitude better than what is indicated in Table III. A more direct approach for improving detection limits is to use larger volumes of sample injected, *e.g.* 10–200 μl. Since the photoionization detector is a concentration-sensitive detector, the use of 50–100-μl sample volumes will lead to detection limits for many compounds in the pg range.

ACKNOWLEDGEMENTS

We wish to thank Dave Locke at the City University of New York for his long-standing interest and contributions to the area of HPLC–PID interfacing and applications. We also express our appreciation for certain reprints and preprints of his own work in this area.

We gratefully acknowledge the donation to Northeastern University of the HNU Systems, PID unit used in all of these studies, as well as other HPLC instrumentation that was used specifically for this program.

This work was supported, in part, by a contract from HNU Systems, to Northeastern University. Additional funding was provided, in part, by a National Institutes

of Health (NIH) Biomedical Research Support Grant No. RR07143 to Northeastern University. This is contribution number 205 from the Institute of Chemical Analysis, Applications, and Forensic Science at Northeastern University.

REFERENCES

1 J. N. Driscoll and F. F. Spaziani, *Res./Dev.*, 27 (1976) 50.
2 J. N. Driscoll, *J. Chromatogr.*, 134 (1977) 49.
3 J. F. Jaramillo and J. N. Driscoll, *J. Chromatogr.*, 186 (1979) 637.
4 J. N. Driscoll, B. J. Grimes and G. F. Hewitt, *Kem.-Kemi.*, 10 (11) (1983) 978.
5 J. N. Driscoll, *J. Chromatogr. Sci.*, 20 (1982) 91.
6 J. N. Driscoll, J. Ford, L. F. Jaramillo and E. T. Gruber, *J. Chromatogr.*, 158 (1978) 171.
7 I. S. Krull, M. Swartz, R. Hilliard, K. H. Xie and J. N. Driscoll, *J. Chromatogr.*, 260 (1983) 347.
8 B. D. Towns and J. N. Driscoll, *Amer. Lab.*, 14(7) (1982) 56.
9 J. N. Driscoll and J. H. Becker, *Pittsburgh Conference, Cleveland, OH, March 1977*, paper No. 387.
10 J. T. Schermund and D. C. Locke, *Anal. Lett.*, 8 (9) (1975) 611.
11 D. C. Locke, B. S. Dhingra and A. D. Baker, *Anal. Chem.*, 54 (1982) 447.
12 I. S. Krull and D. Bushee, *Anal. Lett., Part A*, 13 (1980) 1277.
13 I. S. Krull, E. A. Davis, C. Santasania, S. kraus, A. Basch and Y. Bamberger, *Anal. Lett., Part A*, 14 (1981) 1363.
14 I. S. Krull, M. Swartz and J. N. Driscoll, unpublished results, 1983–1984.