Journal of Chromatography, 83 (1973) 245–254

CHROM. 6789

LIQUID CHROMATOGRAPHY —COLUMNS OR THIN LAYERS?

R. AMOS and S. G. PERRY
Esso Research Centre, Abingdon, Berks. (Great Britain)

SUMMARY

Recent progress in liquid chromatography has developed along two independent lines —thin-layer chromatography, in which the equipment is cheap, simple and easy to use; and the complex, expensive but more powerful technique of high-performance column chromatography, which has recently emerged as a logical extension of the theories and practices developed in gas chromatography. In this paper detailed comparison of the two lines is provided, with particular emphasis placed on cost of equipment, materials and analysis, resolving power, speed and simplicity of the techniques, suitability for automation, accuracy, precision and sensitivity in quantitative analysis and effectiveness in qualitative analysis. It is concluded that the two techniques are complementary; there is a genuine need for both of them and the paper tries to define the areas in which each technique is particularly powerful.

INTRODUCTION

The object of this paper is to provide concrete examples illustrating the advantages and disadvantages of the use of column, instead of thin-layer, liquid chromatography. Although this issue has been discussed, little hard fact has been published hitherto.

The technique of liquid chromatography (LC) made very little progress between its introduction at the beginning of the century and the 1940s although the number of applications increased rapidly during this period. During the past twenty to thirty years, LC has developed along two largely independent lines —firstly, the open-bed techniques of paper and thin-layer chromatography (TLC) in which the apparatus is simple and cheap and secondly, more recently, as a logical extension of the theories and practices developed in gas chromatography (GC), high-performance liquid chromatography in columns (HPLC), in which the equipment is more complex and expensive.

Although TLC and HPLC are basically identical techniques, a wide gulf exists between workers in each individual field, with the result that few objective comparisons have been made. As a consequence, the attractive simplicity of TLC has often been destroyed by attempting to improve its performance either by carrying out a complex operation which cannot easily be controlled, *e.g.* by programming the mobile

and stationary phases, or by the addition of costly ancillary equipment, *e.g.* densitometers for quantitative analysis. In each instance, the extra cost in terms of materials, time and effort is out of all proportion to the extra information gained. On the other hand, it is equally bad practice to tie up the expensive apparatus of HPLC in attacking simple field problems easily solvable with cheap and portable TLC equipment.

At the Esso Research Centre, Abingdon, both GC and TLC have been used extensively for many years with the result that the potentialities of HPLC were recognised at an early stage[1]. So that the two LC techniques are used in the most efficient and effective manner, comparisons have been made in terms of their economics and capabilities and the results are illustrated by typical problems we encounter during the normal workload of our laboratory.

EXPERIMENTAL

Our equipment and techniques are similar to those previously described[1–7]. TLC is carried out either on precoated silica glass plates or on home-made plates coated, *e.g.*, with silica gel G, H or HR with or without fluorescent indicator (E. Merck, Darmstadt, G.F.R.). Development is carried out by the ascending technique either in glass tanks or sandwich chambers (Desaga, Heidelberg, G.F.R.). Separated components are revealed by spraying with one or more of a large number of chromogenic reagents and evaluated either by visible or ultraviolet (UV) light. Quantitative analyses are attempted by densitometry using a Chromoscan densitometer with thin-layer attachment (Joyce, Loebl & Co., Gateshead, Great Britain).

HPLC is carried out in 1 m × 3 mm stainless-steel columns packed with stationary phases of small particles of narrow-size range, typically 20–30 μm. The mobile phase is gravity fed to an Orlita Diaphragm dosing pump Type DMP-1515, typical column inlet pressures being less than 500 p.s.i.g. for a 1-m column. Detection is either by a Model R4 differential refractometer (Waters Ass., Framingham, Mass., U.S.A.) or by a Model CE212 UV monitor (Cecil Instruments, Cambridge, Great Britain). The nominal cell volume in each detector is 10 μl. Stationary phases used include the porous adsorbents Merckosorb SI 60 (E. Merck) and Alumina N18 (Woelm, Eschwege, G.F.R.), the porous layer adsorbents Perisorb A (E. Merck) and Corasil (Waters Ass.), chemically bonded stationary phases for partition chromatography, *e.g.* Durapak (Waters Ass.) and Permaphases (DuPont, Wilmington, Del., U.S.A.), and polystyrene gels for exclusion chromatography, *e.g.* Poragels (Waters Ass.) and Bio-Beads (Bio-Rad Lab., Richmond, Calif., U.S.A.).

RESULTS

Cost, simplicity and convenience

Table I compares the costs of the basic equipment we use for HPLC and TLC. It can be seen that the inclusion of a densitometer increases the capital cost of TLC out of all proportion to the extra information obtained from what is basically a cheap, simple technique involving portable equipment. For quantitative work, a simple, effective and reliable liquid chromatograph can be assembled for about the same cost as the equipment required for quantitative TLC.

Table II compares our liquid chromatograph with its UV detector with a selec-

TABLE I

COST* OF BASIC EQUIPMENT

TLC	*Cost ($)*	*HPLC*	*Cost ($)*
Spreading equipment	150	Pump	1400
Development tank	15	Column	25
Spray gun	10	Detector	2000
Densitometer	3500	Accessories	200
Total	3675	Total	3625

* Typical UK costs at the time of writing.

TABLE II

TYPICAL COSTS OF LIQUID CHROMATOGRAPHS*

Manufacturer	*Description of chromatograph*	*Cost ($)*
Abingdon	Reciprocating pump to 4000 p.s.i. Variable wavelength UV monitor	3700
Waters ALC 202	Piston pump to 1000 p.s.i. Fixed wavelength UV monitor	6000
Hupe Busch UFC 1000	Reciprocating pump to 4000 p.s.i. Fixed wavelength UV monitor Gradient elution Column thermostat	12500
Siemens Model 5200P	Reciprocating pump to 4000 p.s.i. Variable wavelength UV monitor Column thermostat	15500
DuPont Model 830	Single-stroke pump to 3000 p.s.i. Fixed wavelength UV monitor Gradient elution Column thermostat High degree of automation	17700

* Selected to demonstrate points of comparison and do not necessarily imply the chromatograph is the best and only equipment available from a particular manufacturer.

tion of similar commercial chromatographs. It can be seen that laboratory-assembled apparatus possesses the same basic features as a commercial chromatograph, costing two or three times more. It has the added advantages of simplicity and accessibility; also, there are fewer complicating refinements to malfunction whilst columns, mobile phases and detectors can be interchanged within a few minutes. The highest priced chromatographs possess certain additional facilities such as expensive, single-stroke pumps, thermostatted ovens and intricate plumbing arrangements which, though desirable in some instances, are by no means essential. A cheap, reliable and effective means of providing solvent-programming has yet to be devised.

Table III shows the running costs of preparing chromatograms for determining the alkylphenol contents in calcium phenate additives by HPLC and TLC, respectively. The stationary and mobile phases are silica gel and diisopropyl ether in heptane, respectively, and, in the case of TLC, the spots are revealed by spraying with 0.3% *p*-nitrobenzene diazonium fluoroborate in aqueous dioxane, and their spot sizes and intensities measured densitometrically.

TABLE III

ECONOMICS OF ROUTINE ANALYSES

	TLC	*HPLC*
Cost of stationary phase		
for 1 sample	$0.55	$16.50
for 100 samples	$5.50	$16.50
Cost of mobile phase		
for 1 sample	$0.32	$0.32
for 100 samples	$0.64	$16.25
Cost of preparing chromatograms*		
for 100 quantitative analyses	$125.00	$45.00
for 100 semi-quantitative analyses	$45.00	$45.00
Total elapsed time		
for 1 analysis	1.5 h	0.2 h
for 100 quantitative analyses	16 h	16 h
for 100 semi-quantitative analyses	8 h	16 h
Simplicity	Requires skilled operator	Can be automated
Convenience	Portable; does not require laboratory	Requires laboratory

* Labour charge assumed to be $15.00 per hour.

It can be seen that the dearer costs of materials for HPLC are more than offset by the cheaper labour charges. Even semi-quantitative TLC demands considerable skill and patience and therefore requires a higher grade of operator than that needed for the largely automated routine of HPLC.

Although the elapsed time for the analyses of large numbers of samples is similar for both techniques, the elapsed time for a single analysis by TLC is 1.5 h whilst HPLC can produce a result every few minutes. This is obviously advantageous if some process is being continuously monitored. However, if only semi-quantitative comparisons of large batches of samples are required, then TLC becomes the cheapest, simplest and quickest technique. Since the apparatus required for this is cheap and portable, it is ideal for field use as no laboratory facilities are required.

Quantitative analysis

Quantitative analysis by LC can be done more quickly, more cheaply and more precisely by HPLC than by TLC. This is demonstrated by Table IV, which contains comparative data for the determination of the oxidation inhibitor 2,4-dimethyl-6-*tert.*-butylphenol at the 20 mg/l level in naphtha. This analysis is a typical example of the determination of trace amounts of additives in petroleum products. In general, additives have to be concentrated and separated from similar compound types (in this case, naturally occurring phenols) before their concentration can be measured.

The cheaper analyses by HPLC arise, not from a reduction in the cost of equipment and materials, but from the increased sensitivity of the detection methods (*i.e.*, no pre-concentration necessary), a big reduction in the amount of sample handling

TABLE IV

DETERMINATION OF OXIDATION INHIBITORS IN NAPHTHA

	TLC	*HPLC*
Inhibitor level, mg/l	20	20
Detection limit, mg/l	2000	2
Method of detection	Spray with phosphomolybdic acid. Heat 10 min. Scan by densitometry	Continuous UV monitor at 280 nm
Preconcentration	Extraction followed by evaporation	None required
Initial sample size, ml	200	0.1
Stationary phase	Merck precoated silica plate	Merckosorb SI 60, 4% water
Mobile phase	Heptane–diisopropyl ether (80:20)	Heptane–diisopropyl ether (95:5)
No. of standards for 1 sample	5	1
No. of standards for 100 samples	50	2
Analysis time for 1 sample, min	200	10
Analysis time for 100 samples, min	7000	500
Cost of equipment and materials, $	4000	4000
Labour costs* (100 samples), $	1400	100
Standard deviation (2σ), %	3.6	0.8

* Labour charge assumed to be $15.00 per hour.

(*i.e.*, no spraying and heating of plates, no scanning by densitometry), and the increased speed of the chromatographic separation.

The above factors also contribute to the improvement in precision, which is also a consequence of the elimination of two further major sources of error in quantitative TLC. These are the inability to apply, accurately and reproducibly, small volumes of sample to a coated plate and the highly inaccurate and irreproducible method of revealing spots quantitatively by spraying with chromogenic reagents. The improved separation from neighbouring components achieved by the higher efficiency in HPLC is a further factor in improving precision. The fact that quantitative HPLC is a much simpler, mainly automatic technique also means that a lower grade of operator can be employed, not needing the skill and experience demanded by quantitative TLC.

Qualitative analysis

Both TLC and HPLC have important, complementary roles to play in the analysis of an unknown mixture. Briefly, an unknown sample is examined by TLC to get some idea of the complexity of the mixture and, by the use of specific chromogenic reagents, to learn something about the chemical composition of individual components. From the knowledge gained, column systems are devised to effect a more complete separation, by exploiting the higher efficiencies or greater range of selectivities available through the wider range of stationary phases and to collect fractions for other identification techniques. Some points of comparison between the two chromatographic techniques in qualitative analysis are given in Table V.

Fig. 1 illustrates a series of thin-layer chromatograms obtained on a mixed hydrocarbon–synthetic ester based oil containing a range of additives. The chromato-

TABLE V

QUALITATIVE ANALYSIS OF A LUBRICATING OIL

	TLC	*HPLC*
Efficiency, plates sec^{-1}	0.05	1–10
Choice of stationary phase	Mainly limited to adsorption	Free choice of adsorption partition, exclusion or ion exchange
Time to change mobile phase, min	1	30–60
Choice of detectors	Wide range of specific revealing reagents	Restricted choice
Accessibility of chromatogram	Complete	Incomplete and unknown
Time to recover fractions	3 h	5 min

graphic conditions and the conclusions drawn are shown in Table VI. The first advantage of TLC in this preliminary investigation is that the whole of the plate is accessible for inspection, *i.e.* in chromatograms (a) to (f) it is evident that polar material remains completely retained at the starting point, indicating that even more polar mobile phases would be required to elute these components from a column completely. Different mobile phases can be tried at will and several plates can be developed simultaneously. The second advantage of this preliminary TLC investigation is that considerable information is obtained on the probable composition of the sample by the use of specific spray reagents. Both these main features are unique to TLC, and the information gained is vital before HPLC can be successfully applied to an unknown mixture. No matter how sophisticated the instrument, HPLC can be a very disappointing technique if the correct chromatographic conditions are not being used. Changing stationary and mobile phases is tedious and time-consuming, so that in contrast to quantitative work, a higher grade of operator, who understands chromatographic principles, is required for qualitative HPLC.

From the results of the TLC work, high-performance columns can be designed

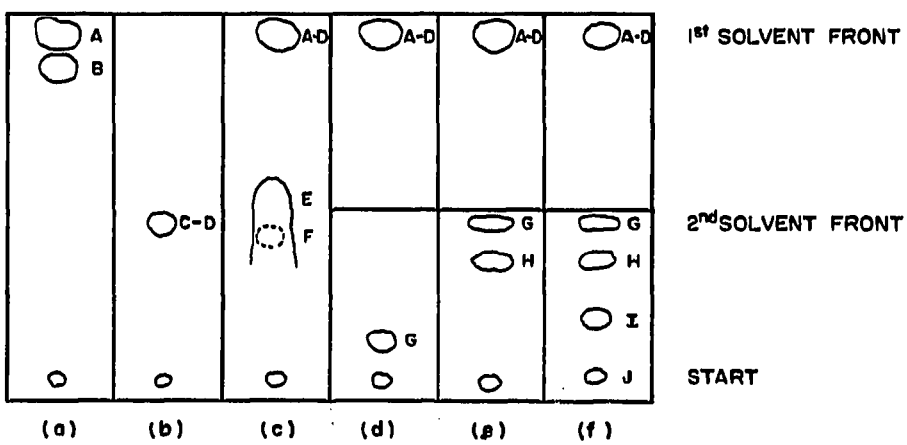

Fig. 1. Qualitative analysis of a lubricant by TLC. For interpretation see Table VI.

TABLE VI
INTERPRETATION OF FIG. 1

Fig. No.	*Mobile phase*	*Revealing method*	*Results*	*Identity*
a	*n*-Heptane	View under long-wave UV light.	A and B show blue fluorescence	Hydrocarbon oil
b	*n*-Heptane	Spray with 1% alcoholic 2,6-dichloroquinone-4-chlorimine.	C and D superimposed brown and violet spots	Dioctyldiphenylamine and octylphenyl-β-naphthylamine
c	Diisopropyl ether–*n*-heptane (30:70)	Spray with 3.5% alcoholic phosphomolybdic acid and heat at 110°	E colourless on yellow background	Synthetic ester
			F not detected	Tricresyl phosphate
			Blue-black spot at start	Polar materials not yet separated
d	Developed 20 cm with toluene followed by second development for 10 cm with *n*-heptane–acetic acid (90:10)	Spray with 0.05% dithizone in carbon tetrachloride	G pink spot	Zinc dialkyldithiophosphate
e	Developed 20 cm with toluene followed by second development for 10 cm with isopropanol–ammonia (80:20)	Spray with diazotised *p*-nitroaniline	H orange spot	Calcium phenate
f	As for (e)	Spray with 0.05% aqueous pinacryptol yellow and view under longwave UV light	I orange fluorescent spot	Calcium sulphonate
			J fluorescence quenching smear	Polymers not separated

to improve the separation of components not resolvable by TLC either by increased efficiency or by use of selective stationary phases.

In Fig. 1b, spots due to dioctyldiphenylamine and octylphenyl-β-naphthylamine are merged whilst the tail of the large synthetic ester spot completely masks the much smaller spot due to tricresyl phosphate. Because of the low rate of plate generation of a TLC plate (0.05 plates sec^{-1}), attempting to increase efficiency by increasing the plate length or by using multi-development procedures would lead to an unacceptably long analysis time because there is no way of controlling the mobile phase velocity. However, Fig. 2 shows the successful separation of all these components within 15 min by using a more efficient column (1–10 plates sec^{-1}).

The second way of improving separation is by use of selective stationary phases. Whilst thin-layer adsorption chromatography is simple to carry out and suitable grades of silica or alumina are readily available and cheap, partition, gel exclusion and ion-exchange chromatography are less easy to perform effectively. As far as we are aware, permanently-bonded partition stationary phases are not available for TLC,

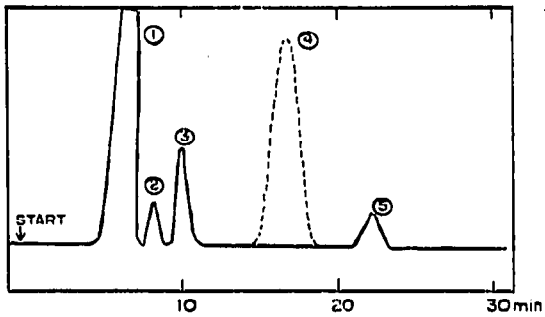

Fig. 2. Separation of low-molecular-weight constituents of a lubricant by HPLC. Chromatographic conditions: Column, 1 m × 4 mm stainless steel; stationary phase, Merckosorb SI 60, 4% water, 20-μm nominal particle diameter; mobile phase, 30% diisopropyl ether in n-heptane at 1.2 ml/min; detection, UV absorbance at 260 nm. Components: 1 = aromatic oil; 2 = dioctyldiphenylamine; 3 = octylphenyl-β-naphthylamine; 4 = synthetic ester (not seen by UV detector); 5 = tricresyl phosphate.

whilst the need to maintain organic gels used for ion-exchange and exclusion chromatography in a swollen state causes practical difficulties with the open-bed technique which are not easily overcome. Stationary phases other than silica and alumina also interfere in the methods of detection, rendering most revealing reagents ineffective. All these disadvantages are overcome in HPLC. Fig. 3, for example, shows the successful separation of two polymeric additives from the oil by gel exclusion chromatography which could not have been achieved by TLC.

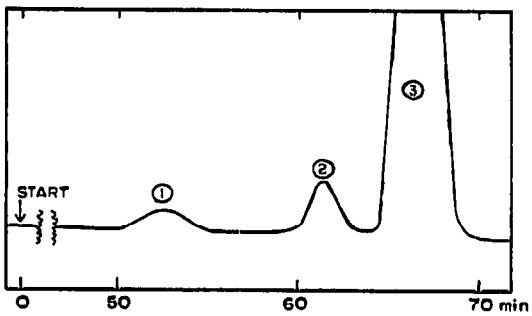

Fig. 3. Separation of high-molecular-weight constituents of a lubricant by HPLC. Chromatographic conditions: column, 3 m × 4 mm stainless steel; stationary phase, Styragel; mobile phase, tetrahydrofuran at 1.3 ml/min; detection, differential refractometry. Components: 1 = high-molecular-weight (500,000) viscosity index improver; 2 = polymeric ashless dispersant; 3 = low-molecular-weight components.

The recovery of fractions for further analysis, *e.g.* for carbon, hydrogen, nitrogen and sulphur contents, for identification by infrared (IR) and mass spectrometry, for quantitative analysis by visible, UV absorption or fluorescence spectrometry or for re-separation GC or alternative HPLC systems, is both quicker and simpler by HPLC than by TLC and purer fractions are obtained. Some pertinent points of comparison are given in Table VII.

TABLE VII

ISOLATION OF PURE COMPONENTS

TLC	*HPLC*
Requires high purity adsorbent	Adsorbent cleaned up *in situ*
Need to prepare a preparative layer	Use existing column
Specialised sampling equipment needed	None needed
Sample application tedious and time consuming	Can be automated
Multiple plates needed for large samples	Repetitive, automatic sample injection on same column
Special precautions needed to protect from oxidation, light and contamination	None needed
Need to locate position of fraction	Automatic, non-destructive detection
Tedious time-consuming sample handling involved in recovering fraction from layer	Automatic. No time element involved
Fraction may be contaminated due to incomplete separation, "dirty" apparatus, and dissolved silica impurities	Purer fractions due to improved efficiencies and reduced sample handling
Typical recoveries: 50–80%	95–100%

Fig. 4 compares IR spectra of the same phenolic antioxidant recovered from a turbine oil by TLC and HPLC, which took 3 h and 5 min, respectively. The presence of impurities in the TLC fraction can be clearly seen, *e.g.* a carbonyl band in the 1700 cm^{-1} region due to contamination by plasticisers from plastic tubing, whilst the spectrum of the material recovered from the column is that of a pure compound.

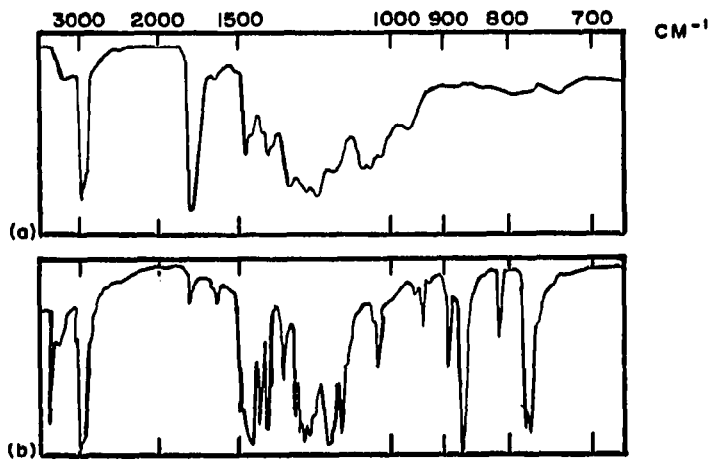

Fig. 4. IR spectra of antioxidant recovered from a turbine oil by (a) TLC and (b) HPLC.

CONCLUSIONS

The comparisons we have made have shown that both TLC and HPLC are needed in our analytical operations and that these two techniques have important complementary roles to play. We use TLC for screening samples of unknown compo-

sition so that suitable column systems can be devised to effect a more complete separation to enable pure components to be recovered for further identification. We also use TLC for semi-quantitative analyses of large numbers of similar samples where a precision of ± 20% of the amount present will suffice. We use HPLC for all routine, high-speed quantitative analyses, covering a concentration range from 10 mg/l to 100%.

REFERENCES

1 H. N. M. Stewart, R. Amos and S. G. Perry, *J. Chromatogr.*, 38 (1968) 209.
2 S. G. Perry, R. Amos and P. I. Brewer, *Practical Liquid Chromatography*, Plenum Publ., New York, 1972.
3 R. Amos, in S. G. Perry (Editor), *Gas Chromatography 1972*, Applied Science Publ., Barking, 1973, pp. 235–245.
4 F. C. A. Killer and R. Amos, *J. Inst. Petrol.*, 52 (1966) 315.
5 R. Amos, *J. Inst. Petrol.*, 54 (1968) 9.
6 R. Amos, *Z. Anal. Chem.*, 236 (1968) 350.
7 R. Amos, *J. Chromatogr.*, 48 (1970) 343.