using porous graphitic carbon. *Journal of Chromatography* 665: 243.

Junk GA, Richard JJ, Grieser MD *et al.* (1974) Use of Macroreticular resins in the analysis of water for trace organic contaminants. *Journal of Chromatography* 99: 745.

Puig D and Barceló D (1995) Off-line and on-line solid-phase extraction followed by liquid chromatography for the determination of priority phenols in natural waters. *Chromatographia* 40: 435.

Puig D and Barceló D (1996) Comparison of different sorbent materials for on-line liquid-solid extraction followed by liquid chromatographic determination of priority phenol compounds in environmental water. *Journal of Chromatography* 733: 371.

Thin-Layer (Planar) Chromatography

J. H. P. Tyman, Institute of Physical and Environmental Sciences, Brunel University, Middlesex, UK

Introduction

Phenols have played an important part in thin-layer chromatography (TLC) since its inception as a general separatory method. The phenolic group occurs in a wide variety of natural replenishable organic compounds from the amino acids (tyrosine), the alkaloids (morphine), the steroids (estrone), glycosides (arbutin, carminic acid, flavonols), perfume ingredients (methyl salicylate, eugenol), phenolic lipids (cashew phenols, urushiol) and the tetracyclines to the cannabinoids. Fractions derived from fossil fuels by distillation or by synthesis contain phenol itself, the cresols, xylenols and polynuclear compounds. Many purely synthetic products, notably drugs (aspirin, paracetamol), technical products (*t*-butylphenol, nonylphenol, and other intermediates such as chloro- and nitrophenols extend the range of substances as candidates for TLC.

The classical separations of structural isomers of monohydric, dihydric and polyhydric phenols and their derivatives have generally formed the basis for subsequent applications to mixtures of more complex compounds. All these initial studies were essentially qualitative and have led over the past four decades to quantitative work aimed at the determination of a single phenolic substance in a mixture or of the total phenolic composition.

The original TLC adsorption method with silica gel was soon supplemented by the partition technique by coating the adsorbent with a paraffinic compound or by silanization. These reversed-phase (RP) approaches have, with the introduction of excellent commercial plates, led to the routine use of alkylsilyl-bonded silica gel with C_{18}, C_8 or C_2 silyl groups.

In a similar fashion, with the development of uniform particle size adsorbents high performance TLC (HPTLC), combined techniques such as TLC–ultraviolet (TLC-UV), TLC–mass spectrometry (TLC-MS), (TLC-GLC) and TLC–high performance liquid chromatography (TLC-HPLC) have all been applied to the study of phenolic materials.

A wide variety of adsorbent layers have been investigated for the analysis of phenols, including silica gel, alumina, cellulose and polyamides as well as mixtures in certain cases with other inorganic materials such as calcium hydroxide or impregnated with organic additives. The use of argentation TLC with phenolic lipids is well established but other ions, for example Fe(III) and Cu(II), have found limited application.

Solvents from the whole elutropic range have been employed and frequently basic conditions have been found advantageous by the use of ammonia or by the addition of an organic base, although solvents acidified with formic acid have an application. With the availability of silica gel G_{254}, visualization of phenolic substrates is straightforward, although with non-indicating silica gel, the use of dichlorofluorescein, rhodamine 6G or a wide selection of spray reagents has become common practice and almost completely displaced the use of sulfuric acid and thermal charring. Documentation by means of photocopying, photography or by the use of dyeline paper and an ammoniated atmosphere are all effective methods.

In the following examples, the analytical scale TLC separations of a range of isomeric compounds are tabulated. At the preparative scale increased sample loading leads to a loss of resolution and indeed the loading/resolution factor should be examined in all cases.

Separation of Structural Isomers of Phenols and Phenolic Acids

The R_F values of an extensive range of phenolic compounds of synthetic and natural origin have been given in the literature listed in the Further Reading

section, notably in the Tables in Tyman (1996) and Hanai (1982).

Cresols

The separation of structural isomers represents an effective test of a chromatographic method and this is particularly relevant to TLC where a considerable body of work has been devoted to the *o*-, *m*- and *p*-derivatives of phenol. Thus, in **Table 1** some results, from experiments covering a period of nearly four decades, are shown on the cresols. Marginal differences of R_F values are seen in solvent systems a–f, h, and less so in i. Remarkably, it is only recently that an acceptable separation has been achieved with system g involving silica gel G, although in this case a second run would be desirable if phenol itself were present in a commercial sample.

Ethylphenols

The *o*-, *m*- and *p*-isomer of the homologous ethylphenol might similarly be separated. **Table 2** indicates four other systems which have been examined and only in system a with alumina is the separation well defined. A crucial factor of importance in isomer separations is the relative R_F values of the *m*- and *p*-isomers since a steric influence aids the differentiation of the *o*-isomer.

In the case of higher homologues, for example the isomeric *t*-butylphenols, silica gel (type 60) with benzene appears to be marginally more effective than alumina with diisopropyl ether or cellulose (by RP with aqueous ethanol), giving for the *o*-, *m*- and *p*-isomers hR_F values of 54, 22 and 16 respectively.

Chlorophenols

The hR_F values of the isomeric monochlorophenols are shown in **Table 3**. This series has been widely studied by adsorption methods (b–e, g) and by partition procedures (a, f, h) and, generally, greater resolution has been found with the latter. Nevertheless, by the use of basic layers (b) or with basic solvents (c) almost comparable results are achieved. As with many isomeric compounds, the 3- and 4-isomers present the main problem.

Table 2 hR_F Values of phenol and isomeric ethylphenols on various layers

Compound	*Conditions*			
	a	*b*	*c*	*d*
Phenol	13	54	73	79.5
2-Ethylphenol	32	77	25	40
3-Ethylphenol	14	—	32	48.5
4-Ethylphenol	24	61	28	45.5

[a]Tyman (1996a): diisopropyl ether, alumina$_{254}$. [b]Hanai (1982): benzene–ethyl acetate, 95 : 5, silica gel FG. [c]Tyman (1996a): 8 mol L^{-1} ammonia in 20% methanol, silanized silica gel impregnated with triethanolamine salt. [d]Tyman (1996a): ethanol–water, 75 : 25, cellulose, reversed phase.

Nitrophenols

The separation of the isomeric nitrophenols depicted in **Table 4** presents relatively little difficulty and generally adsorption conditions (b–e) have proved as effective as others; the use of impregnated silanized silica gel (f) does not seem to aid the *o*-/*p*- compound separation. The increased polarity of the nitro group compared with the chloro substituent appears to help the separation of the 3- and 4-isomers.

Table 1 hR_F Values of phenol and isomeric methylphenols (cresols) on various layers

Compound	*Conditions*								
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
Phenol	43	63	46	34	30	63	37	63	79
o-Cresol	52	92	60	33	31	74	53	50	55
m-Cresol	45	84	51	29	38	63	36	50	63
p-Cresol	49	79	49	31	33	65	46	55	57

[a]Petrowitz (1969): benzene–methanol, 95:5, silica gel G. [b]Hanai (1982): benzene–methanol 90 : 10, alumina–calcium hydroxide 40 : 20. [c]Truter (1963): chloroform, silica gel G–starch. [d]Hanai (1982): 20% methyl-*t*-butyl ether in hexane, silica gel G. [e]Bund *et al.* (1995): methyl-*t*-butyl ether–hexane, 10 : 90, silica gel RP-18. [f]Petrovic *et al.* (1992): silica gel impregnated with Cu(II) and with Al(III), chloroform–acetone, 95 : 5. [g]Baranowska and Skotniczna (1994): chloroform–isopropanol, 49 : 1.5, silica gel G. [h]Baranowska and Skotniczna (1994): benzene–isopropanol, 50 : 4. [i]Tyman (1996a): 8 mol L^{-1} ammonia in 20% methanol, silanized silica gel impregnated with dodecylbenzene sulfonate triethanolamine salt.

Table 3 hR_F Values of phenol and isomeric chlorophenols on various layers

Compound	*Conditions*							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
Phenol	30	66		16	47	73	79	
2-Chlorophenol	48	30	21	42	50	93	53	77
3-Chlorophenol	34	20	34	20	47	82	43	50
4-Chlorophenol	28	27	41	16	43	67	46	37

[a]Bund *et al.* (1995): methyl-*t*-butyl ether–hexane, 10 : 90, silica gel RP-18. [b]Hanai (1982): benzene–methanol, 90 : 10, alumina–calcium hydroxide. [c]Hanai (1982): toluene–piperidine, 5 : 2, silica gel GF_{254}. [d]Tyman (1996a): benzene, silica gel G. [e]Tyman (1996a): diethyl ether–*n*-hexane, 1 : 1, silica gel G. [f]Tyman (1996a): system 5A, 1 M (pH 11.30) ammonia in 30% methanol, silanized silica gel. [g]Tyman (1996a): ethanol–water, 75 : 25, cellulose, reversed phase. [h]Lepri *et al.* (1982): 1 M (pH 11.30) ammonia + 0.1 mol^{-1} ammonium acetate in 20% methanol, silica gel RP-18, impregnated with 4% dodecylbenzene sulfonic acid.

Aminophenols

The isomeric aminophenols have been separated as shown in **Table 5** on a diverse range of adsorbents and solvent systems, all of which have yielded acceptable separations, although system b, one of the most recently examined, is to be preferred above the others.

Dihydroxybenzenes

In a similar way, the dihydroxybenzenes have been studied widely and the same system as used for the aminophenols (**Table 5**, condition a) has afforded the best separation of the three isomers, as depicted in **Table 6**.

Hydroxybenzaldehydes and Hydroxyketones

In the carbonyl series, comparatively little work has been carried out on either the hydroxybenzaldehydes or ketones. **Table 7** indicates that no single system effects the separation of all three isomers and the use of system a with e would be desirable.

In the ketone series, 2- and 4-hydroxybenzophenones appear to be readily separable from benzophenone itself on silica gel GF in benzene, with hR_F values of 53, 0 and 63 respectively. In benzene–piperidine (9 : 1), the corresponding values were 59, 9 and 77.

Phenolic Acids

Experimentation on the separation of isomeric phenolic acids has been widespread and a number of systems have been introduced, as shown in **Table 8**. The prevalent use of mildly acidic or basic solvent systems is seen compared with previous separations. In system a the formic acid enhances the separation of the 2-isomer through increased H-bonding, while in b–d the use of a basic solvent generally improves the resolution of all the isomers. The acetic acid component of systems e and f is insufficient to influence the separation of the 3- and 4-isomers.

Table 4 hR_F Values of nitrophenols on various layers

Compound	*Conditions*					
	a	*b*	*c*	*d*	*e*	*f*
Phenol	30		48	29	74	
2-Nitrophenol	64	96	75	57	79	83
3-Nitrophenol	20	80	43	14	62	68
4-Nitrophenol	12	65	35	8	46	89

[a]Bund *et al.* (1995): diethyl ether–*n*-hexane, 1 : 1, silica gel G. [b]Hanai (1982): benzene–ethyl acetate, 90 : 10, zinc carbonate–silica gel, 30 : 20. [c]Hanai (1982): benzene–methanol, 95 : 5, silica gel G_{254}. [d]Hanai (1982): 20% diisopropyl ether in hexane, silica gel 60. [e]Tyman (1996a): diisopropyl ether, silica gel 60. [f]Tyman (1996a): system 4B.

Some Qualitative Separations of Mixtures of Phenolic Derivatives

Natural Phenolic Acids

Many natural phenolic acids have a useful pharmacological activity and plant sources containing them have a phytotherapeutic function. The two-dimensional TLC separation of reference mixtures or of the plant extracts of *Polygonum amphibium* has been effected first by development with benzene–methanol–acetonitrile-acetic acid (80 : 10 : 5 : 5) and, after removal of the eluent by evaporation,

Table 5 hR_F Values of isomeric aminophenols on various layers

Compound	*Conditions*							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
Phenol	32	90	91	58	22	19		
2-Aminophenol	53	50	68		31	34	30	55
3-Aminophenol	63	37	57	80	39	43	25	49
4-Aminophenol	69	14	52	86	53	50	19	41

[a]Bund *et al.* (1995): methanol–water, 50 : 50 RP-18. [b]Bund *et al.* (1995): hexane–methyl *t*-butyl ether, 40 : 60. [c]Petrovic *et al.* (1992): toluene–chloroform–acetone, 40 : 25 : 25, silica gel GF + Cu(II) or Al(III). [d]Tyman (1996a): 0.21 mol L^{-1} NaCl–formamide–acetonitrile, 41 : 15 : 44, silica gel C_{18}. [e]Hanai (1982): 0.5 mol L^{-1} ammonia in 50% ethanol, Biorad AG3-X4A. [f]Hanai (1982): water–methanol, 4 : 1, BD-cellulose. [g]Hanai (1982): benzene–methylethyl ketone, 90 : 10, zinc carbonate–silica gel 30 : 20. [h]Hanai (1982): benzene–methanol, 95 : 5, silica gel GF.

a second development at right angles is carried out with sodium formate–formic acid–water (10 : 1 : 200, w/v/v). The result of the first development is shown in **Figure 1A** and of a second development in **Figure 1B**. The formulae of the 14 acids are shown in **Figure 2**.

Methylolation of 3-Pentadecylphenol

The methylol derivatives of 3-pentadecylphenol are of interest for the solvent extraction of the borate ion and are obtained by the methylolation of 3-pentadecylphenol with formaldehyde, a reaction which affords mainly the 4- and 6-methylols, a minor proportion of the 2-isomer, the 2,4-, 2,6- and 2,4-dimethylols and some 2,4,6-trimethylol. The reaction mixture of isomeric compounds has been separated on silica gel G with light petroleum (60–80°C)–diethyl ether–dimethylformamide–acetic acid (75 : 85 : 5 : 1), as depicted in **Figure 3**. It is imperative to confirm the identity of bands by spectroscopic means and by the use of synthetic reference compounds in the 2-, 4- and 6-methylol series obtained in the present instance by reduction of the corresponding phenolic acid or aldehyde.

Table 6 hR_F Values of dihydric phenols on various layers

Compound	*Conditions*					
	a	*b*	*c*	*d*	*e*	*f*
Phenol	32	63	74	36	59	
1,2-Dihydroxybenzene	47	40	49		29	45
1,3-Dihydroxybenzene	55	30	39	58	36	40
1,4-Dihydroxybenzene	64		43	67	48	32

[a]Bund *et al.* (1995): methanol–water, 50 : 50 RP-18. [b]Baranowska and Skotniczna (1994): benzene–isopropanol, 50 : 40, silica gel G. [c]Tyman (1996a): diisopropyl ether, silica gel 60. [d]Tyman (1996a): system 1A, silanized silica gel. [e]Hanai (1982): biorad AG3,X4A, 0.5 ammonia in 95% ethanol. [f]Hanai (1982): benzene–ethyl acetate, 90 : 10, zinc carbonate–silica gel, 30 : 20.

Phenolic Components and Constituents of *Anacardium occidentale* (Cashew)

Mixtures of phenols and phenolic acids such as occur in the shell of the cashew are used as precursors for the manufacture of certain formaldehyde resins. They have been separated with diethyl either–light petroleum (40–60°C)–formic acid (30 : 70 : 1), as shown in **Figure 4** on silver-impregnated silica gel G, which also affords a separation of the saturated, 8Z-monoene, 8Z,11Z-diene and 8Z,11Z,14-triene constituents of each component phenol. In the absence of silver ion, the four main component phenols cardol, 2-methylcardol, cardanol and anacardic acid (each containing four constituents, $n = 0, 2, 4, 6$) have hR_F values 20, 36, 58 and 76 respectively. By contrast, in an ammoniated solvent with multiple development,

Table 7 hR_F Values of isomeric hydroxybenzaldehydes on various layers

Compound	*Conditions*					
	a	*b*	*c*	*d*	*e*	*f*
Phenol					26	
2-Hydroxybenzaldehyde	63	52	63	71	59	70
3-Hydroxybenzaldehyde	79	57	69	78	9	66.5
4-Hydroxybenzaldehyde	64	56	68	76		66.5

[a]Tyman (1996a): 2% formic acid. [b]Tyman (1996a): 20% KCl. [c]Tyman (1996a): iPrOH–NH_4OH–H_2O, 8 : 1 : 1. [d]Tyman (1996a): 10% AcOH. [e]Hanai (1982): silica gel 60F, 20% Et_2O–hexane. [f]Hanai (1982): polyamide, butan-2-one–acetophenone–50% acetic acid, 5 : 5 : 4.

Table 8 hR_F Values of isomeric phenolic acids on various layers

Compound	*Conditions*					
	a	*b*	*c*	*d*	*e*	*f*
Benzoic acid		70		53		
2-Hydroxybenzoic acid	70	82	63	55	59	75
3-Hydroxybenzoic acid	37	59	21	31	39	67
4-Hydroxybenzoic acid	27	50	34	17	38	66

[a]Tyman (1996a): 2% formic acid, cellulose. [b]Tyman (1996a): ethanol–ethyl acetate–ammonia, 9 : 3 : 2, silica gel G impregnated with silver oxide. [c]Hanai (1982): isopropanol–benzene–conc. ammonia, 3 : 1 : 1. [d]Hanai (1982): ethanol–butanol–water–conc. ammonia, 49 : 30 : 15 : 15, rice starch. [e]Hanai (1982): upper phase of benzene–acetic acid–water, 10 : 15 : 5, silica gel. [f]Hanai (1982): toluene–acetic acid–water, 70 : 20 : 1, silica gel G.

a system used subsequently for quantitative analysis and described later, the phenolic acid anacardic acid was retained at low hR_F and separated from the three other phenols.

Resorcinolic Compounds from Rye

The homologous cardol constituents in rye (*Cereale scale*) have been separated by the use of a two-way development involving a preliminary separation (direction 1) of the saturated, monoene and diene members on argentated silica gel with benzene–ethyl acetate (85 : 15) and then, after removal of the silver ion and impregnation of the silica gel with paraffin oil, RP separation of the C_{13} to C_{29} homologues by development (direction 2) with acetone–methanol–water (60 : 15 : 25), as shown in **Figure 5**.

Factors Influencing the Separations of Phenolic Compounds

Although, as with many classes of organic compound, the nature of the adsorbent, of the functional groups and of the position of the solvent or solvent mixture in the relative elutropic series are dominant influences, phenols have a unique property in respect of the intramolecular hydrogen bonding of a considerable number of the *o*-functional groups with the hydroxyl group. The hR_F values of *o*-substituted phenols on silica gel under adsorption conditions extend over a wider numerical range than those of the corresponding *m*- and *p*-isomers. Strongly hydrogen-bonding groups such as NO_2, CHO and Cl head the series, as do bulky alkyl groups which by contrast exert a purely steric effect.

This is seen particularly with 2,6-compounds with nonpolar substituents such as the 2,6-di-*t*-butyl derivatives which are important antioxidants. These very nonpolar compounds are also actually less polar than their methyl ethers. **Table 9** depicts the hR_F values for a number of 2,6-disubstituted compounds. A steric factor operates in the 2,6-dialkylphenol series, where 2,6-dimethyl, 2,6-diethyl, 2,6-diisopropyl, 2,6-diallyl and 2,6-di-sec-butylphenol have hR_F values of 43, 63, 86, 54, 94 respectively compared with phenol, 9, and the 2,6-di-*t*-butylphenol, 94, on cellulose plates

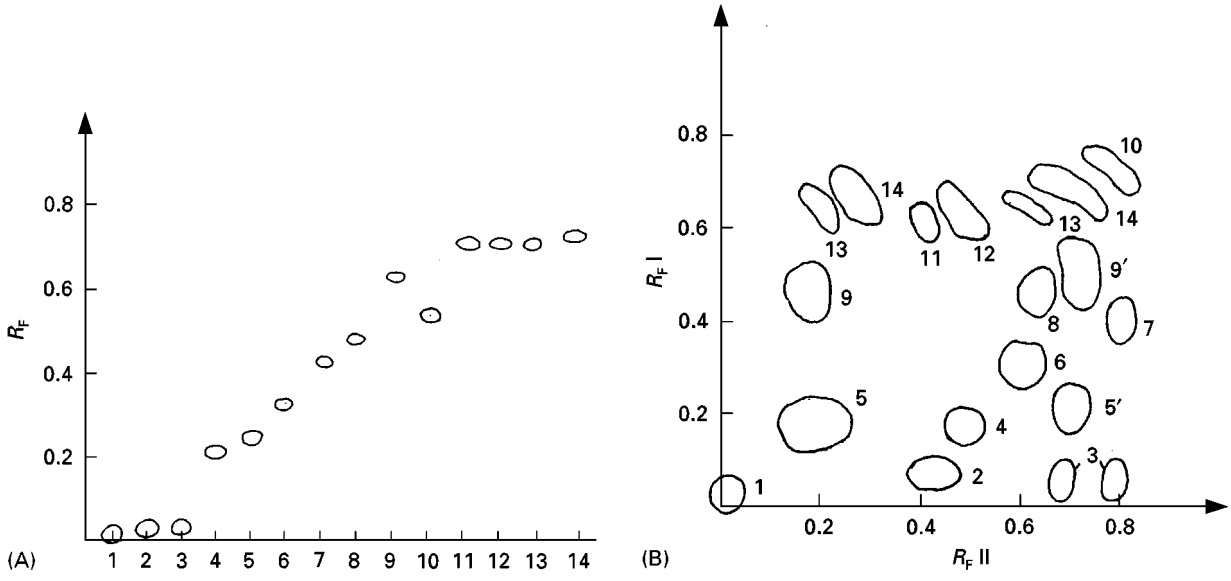


Figure 1 (A) One-dimensional separation of phenolic acids 1–14 (formulae as in Figure 2). 5′ and 14′ represent *cis* isomers. (Reproduced with permission from Smolarz and Waksmundzka-Hajnos, 1993.) (B) Two-dimensional separation of phenolic acids 1–14. (Reproduced with permission from Smolarz and Waksmundzka-Hajnos, 1993.)

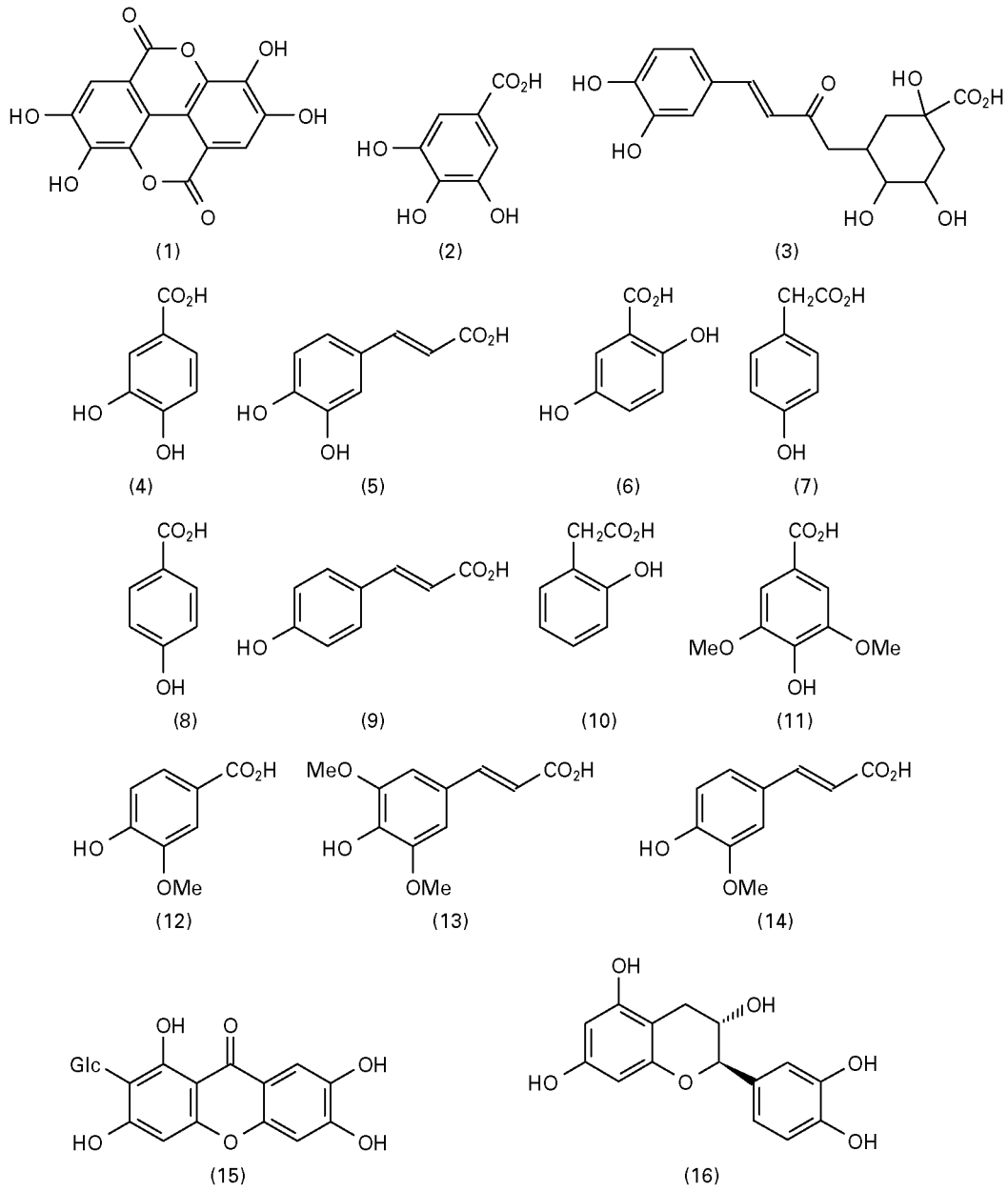


Figure 2 Formulae of phenolic acids 1–14.

impregnated with *N*-methylformamide and developed in hexane (Table 9).

With the *m*- and *p*-series, the hR_F values of all compounds are nearly parallel. The NO_2, CHO and Cl groups now simply exert a polar effect (and an electronic effect on the phenolic acidity) while alkyl groups have a minor steric role. The isomeric phenolic acids behave similarly with respect to hydrogen bonding. 2,3- 2,4- and 2,5-Disubstituted compounds compare with the monosubstituted 2-series while 3,4- and 3,5-disubstituted compounds compare with 4-monosubstituted phenols.

Although current theory has no predictive use for structural assignment of polysubstituted compounds from their hR_F values, nevertheless the vast number of such values available from the literature affords a guide to the selection of an appropriate system. There is little doubt that solvent selection is the most important influence, as illustrated in **Table 1** where, with virtually the same adsorbent in entries (c) and (g), a period of 40 years separates a relatively poor from an excellent resolution, obtained simply by the addition of isopropanol to the same solvent.

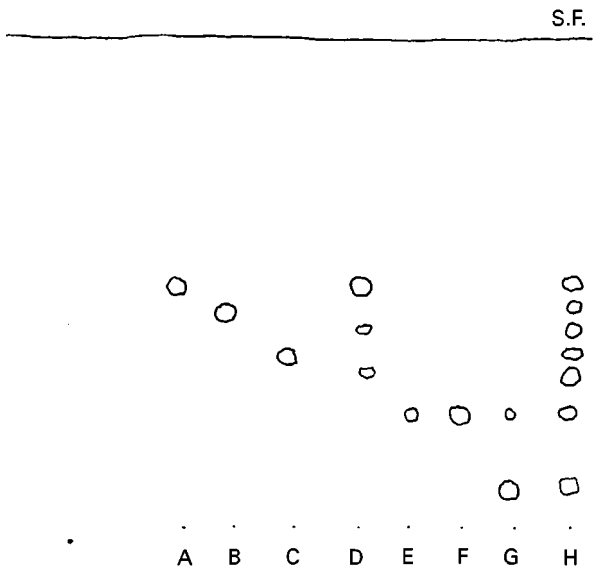


Figure 3 Separation of methylol derivatives of 3-pentadecylphenol. A, 2-methylol; B, 6-methylol; C, 4-methylol; D, mixture of 2-methylol, 2,6-dimethylol and 2,4-dimethylol; E, 4,6-dimethylol; F, 4,6-dimethylol; G, 2,4,6-trimethylol; H, mixture of all the synthetic methylols. (Adapted with permission from Tyman, 1996a.)

This crucial role of the solvent is seen in the R_F values of certain hydroxyanthraquinones, depicted in **Figure 6**, where the advantage of hydrogen bonding in affording a separation of compounds 1 and 2 from 3 and 4 is sacrificed with the use of the polar solvents *n*-butanol or ethyl acetate. It is perhaps relevant to reiterate the value of the Stahl triangle (**Figure 7**) for separations to be effected under adsorption conditions.

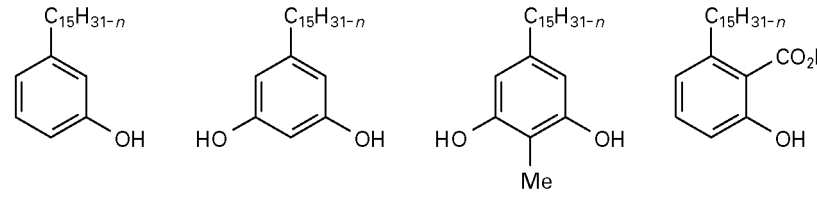
Quantitative Separation of Mixtures of Phenolic Compounds

A variety of applications of TLC to the analysis of phenolic compounds in biochemical, environmental, industrial and consumer products has been summarized by Tyman (1996). In the following descriptions a selected range of quantitative studies are given. In the first the phenolic components of natural cashew nut-shell liquid (CNSL), comprising anacardic acid, cardol, 2-methylcardol and cardanol, have been determined by the preferred method of densitometry, 'on the plate' and also by UV spectrophotometry 'off the plate'. The natural product is essentially a mixture of a phenol, a resorcinol, a hindered resorcinol and a phenolic acid. Its composition is required to assess the effectiveness of its industrial thermal decarboxylation at 200°C for obtaining technical CNSL, a product widely used in friction dusts for use in automobile brake and clutch systems.

Composition of Natural Cashew Nut-shell Liquid from *Anacardium occidentale* by Densitometry

Following lengthy experimentation, multiple development was used, initially with light petroleum (40–60°C)–diethyl ether–ammonia solution (50 : 50 : 5) to separate cardanol, 2-methylcardol from cardol/ammonium anacardate and then with diethyl ether–ammonia (90 : 10) to separate cardol and ammonium anacardate. The hR_F values were ammonium anacardate (0), cardol (36), 2-methylcardol (61) and cardanol (71). Subsequently, for the analysis of technical CNSL, which contains predominantly cardanol, cardol and 2-methylcardol, the alternative solvent system light petroleum–diethyl ether–formic acid (70 : 30 : 1) was employed, in which the hR_F values are 58, 20 and 36 respectively.

The natural product was obtained from frozen raw cashew nuts by cracking followed by solvent extraction and recovery at ambient temperature. Commercial silica gel G plates (20 × 20 cm) were used and multiple development in an ammoniated solvent system with 10, 20 and 30 μL of the unknown sample applied alongside 10, 16, 20, 24 and 30 μL of a standard solution containing anacardic acid, cardol, 2-methylcardol and cardanol. After development, removal of the solvent, visualization with iodine, the use of a cover plate and both horizontal and vertical scanning, the areas of all the bands were obtained by integration. A typical plate is illustrated in **Figure 8**.



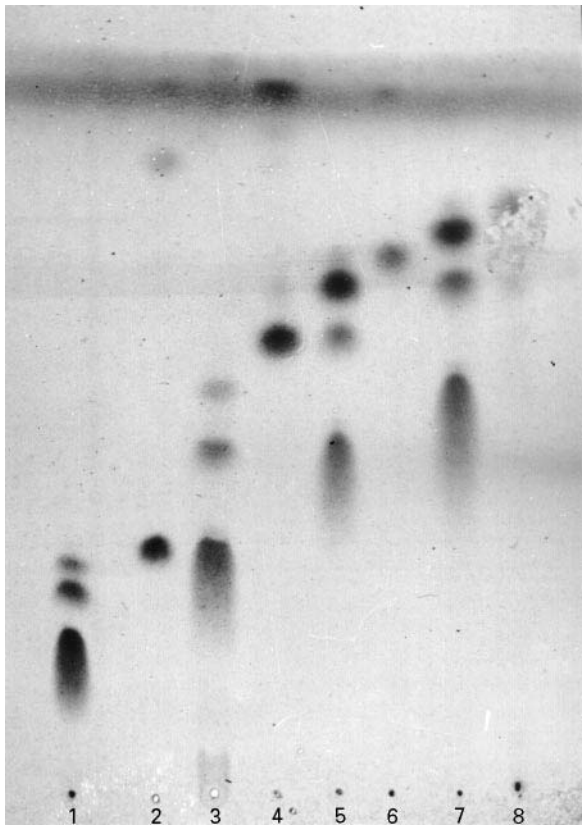


Figure 4 TLC of cashew phenols before and after hydrogenation on silica gel G with 10% silver nitrate, Samples 1, 3, 5 and 7 are cardol, 2-methylcardol, cardanol and anacardic acid. Samples 2, 4, 6 and 8 are the hydrogenated samples showing absence of unsaturated constituents. Detection with sulfuric acid and charring. (Adapted with permission from Tyman, 1996a.)

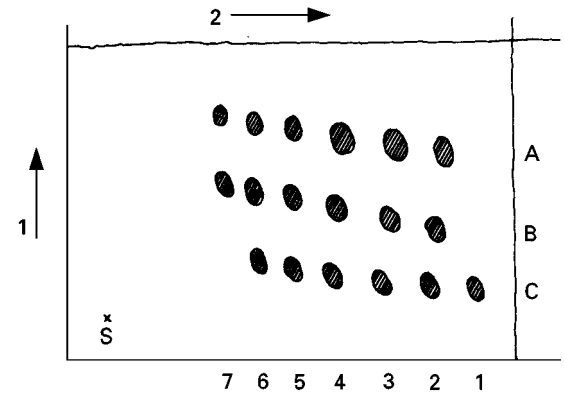


Figure 5 Two dimensional separation of 5-alkenylresorcinol homologues. Development 1 was first to 5 cm and then to 8 cm. Development 2 was at right angles. Detection was with Fast Blue B. A, saturated; B, monoene, C, diene constituents; S, starting point. Numbers 1–7 represent C_{13} to C_{25} respectively. (Adapted with permission from Tyman, 1996a.)

Plots of area by densitometry versus volume (μL) of the standard solution for each standard phenolic component gave a series of straight lines conforming to the relation $y = mx$, to which the least-squares method was applied in order to determine accurately the slope m.

From the resultant plots and the areas by densitometry, the volumes (equivalent μL) of the unknown solution were ascertained. By simple proportionation relative to the known weights of the four phenols in the volume of standard solution used, the weights of the four component phenols in the unknown were then found. **Table 10** depicts some results of two runs from a total of nine experiments. The average recovery (natural CNSL found/weight taken) was 98.0%.

Elution and Indirect Spectrophotometry

In the 'off the plate' method the same ammoniated system was used with multiple development, the bands were visualized with Rhodamine 6G, removed from the plate and then eluted to recover the phenolic components which, after freeing from indicator by acidification, were made up to standard volume and examined by UV spectrophotometry. From the epsilon values [(E 1% 1 cm × mol. wt)/10] found, after correction for the purity of the phenols from the values of pure standards, the weights of the four component phenols were obtained and hence the percentage composition. The UV absorption of the four phenols is depicted in **Figure 9**.

It is of interest to compare the compositional analyses of natural CNSL by densitometry, gas chromatography (GC) and high performance liquid chromatography (HPCL) as depicted in **Table 11**.

Table 9 hR_F Values of hindered phenols and their methyl ethers on silica gel G in light petroleum, 40–60°C (LP)/chloroform (C)

Compound	*Conditions*				
	a	*b*	*c*	*d*	*e*
2,6-Di-*t*-butyl-4-methylphenol	36	64	75	83	84
2,6-Di-*t*-butyl-4-methylanisole	27	57	75	83	84
2-*t*-Butyl-6-methoxy-4-methylphenol	12	31	44	66	76
2-*t*-Butyl-6-methoxy-4-methylanisole	4	20	32	55	66
2-Methoxy-4,6-di-*t*-butylphenol	14	41	52	68	73
2-Methoxy-4,6-di-*t*-butylanisole	8	29	38	57	64
2-Methoxy-6-pentadecylphenol	11	29	38	60	70
2-Methoxy-6-pentadecylanisole	65	17	24	48	65

Adapted with permission form Tyman 1996a. [a]Lp 100; [b]LP-C, 80 : 20; [c]LP-C, 60 : 40; [d]LP-C, 40 : 60; [e]100.

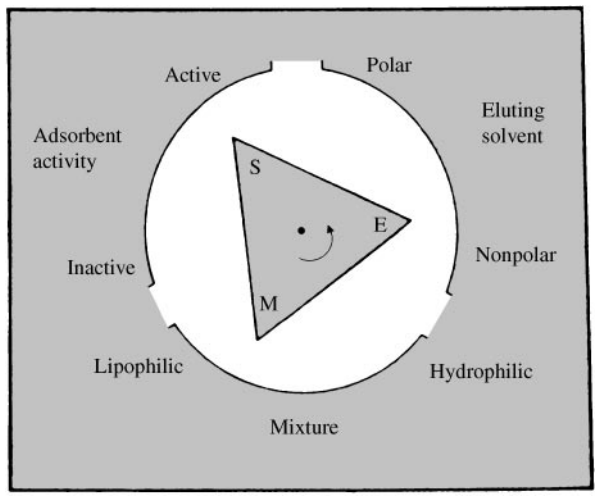


Figure 6 Separation of hydrogen-bonded derivatives of 1,8-dihydroxy and of 1-hydroxyanthraquinones. S, starting point; M, mixture; E, eluant. (Reproduced with permission from Tyman and Morris, 1967.)

TLC methods have also been employed with the phenolic lipid urushiol from *Rhus vernicifera*.

Determination of Phenolic Compounds, Mangiferin, Colladin and Colladonin in *Colladonia triquetra*

The natural product mangiferin (15), 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthene-9-one, which posesses mono hydrogen-bonded resorcinol and catechol moieties in its structure, occurs as the major component in the Bulgarian medicinal plant *Colladonia triquetra* and is useful for cardiotonic, spasmolytic, diuretic and in other phytotherapeutic applications. In connection with agricultural production an improved analysis was developed for mangiferin by TLC and compared with an HPLC method. Colladin and colladonin are minor components in the natural product extracted.

Seeds of the natural product (2 g) were extracted with hot methanol and after recovery by concentration the residual material was dissolved in dioxan–water (1 : 1) filtered and then made up to a volume of 25 cm^3, from which an aliquot was diluted 10-fold with dioxan–methanol (1 : 1) to give solution II. A standard solution of pure mangiferin (0.0100 g) was prepared in dioxan–methanol (1 : 1) (100 cm^3).

Silica gel 60 F_{254} plates were used and 1.0, 2.0, 3.0 and 4.0 μL of solution II applied alongside 5.0, 6.0, 7.0, 8.0 and 9.0 μL of the standard solution of pure mangiferin and developed with the solvent ethyl acetate–formic acid–water (67 : 13 : 20) to a distance of 10 cm. After drying, the bands were scanned for their fluorescence under UV illumination with Helena Laboratories Cliniscan densitometer and quantification followed from the peak areas obtained by integration. In a similar way, using standard solutions of colladin and colladonin alongside solutions of the natural extract with the solvent benzene–methyl ethyl

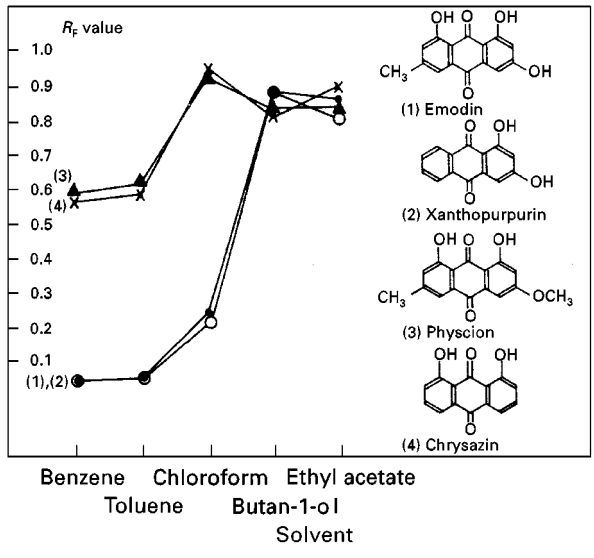


Figure 7 The Stahl triangle. Open circles, (1) emodin; closed circles, (2) xanthopurpurin; triangles, (3) Physcion; crosses, (4) chrysazin. (Reproduced with permission from Tyman and Morris, 1967.)

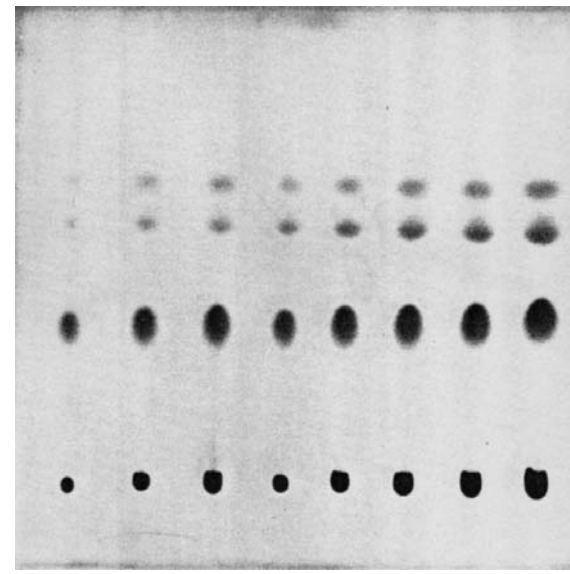


Figure 8 Separation of cashew phenols in an ammoniated system. R_F values of cardanol, 2-methylcardol, cardol and ammonium anacardate in descending order and from left to right, three samples of the unknown and then five of a standard solution. (Reproduced with permission from Pusey, 1969.)

Table 10 Percentage composition of component phenols in natural cashew nut-shell liquid (CNSL) by 'on the plate' densitometry

Vol. CNSL Standard (μL)	*Equiv. μL from area/volume plots*				*wt. (g) component phenol*				*Total (g)*
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	
20	12.16	13.20	5.88	8.76	0.1442	0.0420	0.0060	0.0098	0.2020
%					71.38	20.79	2.97	4.86	
20	12.08	13.60	6.24	9.04	0.1432	0.0432	0.0064	0.0101	0.2029
%					70.57	21.31	3.13	4.99	
Average of 7 runs					71.33	20.28	3.25	5.12	

[a]Anacardic acid; [b]cardol; [c]2-methylcardol; [d]cardanol. Adapted with permission from Pusey (1969).

ketone–formic acid (9 : 1 : 1) and densitometric evaluation as before, their percentage occurrence in the natural product was determined. Comparison with an HPLC method based on the use of LiChrosorb RP-18 was made and the results are shown in **Table 12.**

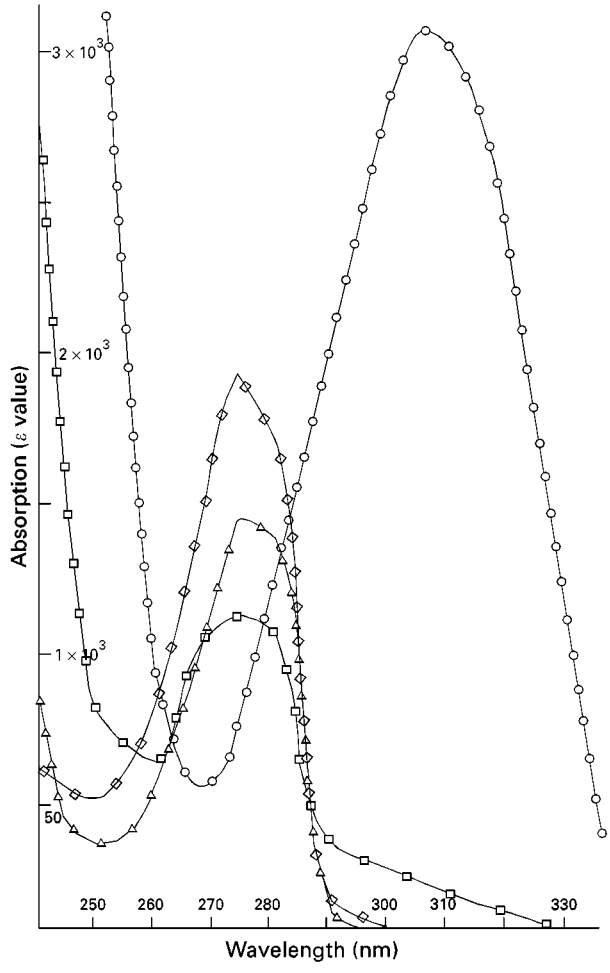


Figure 9 UV absorption spectra of the component phenols of natural cashew nut-shell liquid. circles, Anacardic acid; squares, cardanol; triangles, cardol; diamonds, 2-methylcardol. (Reproduced with permission from Pusey, 1969.)

Determination of the Flavan, Catechin in Cube Gambir, from *Uncaria gambir*

Cube gambir, which is obtained industrially by drying the aqueous extract of twigs and leaves of *Uncaria gambir*, has uses in dyeing and tanning, as an astringent and more recently for its antiulcer activity attributed to its component catechin, (2*R*,3*S*)-(+)-3,3′,4′,5,7-pentahydroxyflavan, a compound with resorcinol and catechol structural groupings. It has to be distinguished from black catechu, which contains (2*R*,3*S*)-(−)-epicatechin and for its analysis TLC and HPLC methods have been evaluated.

With commercial HPTLC plates of silica gel 60 F_{254} and the solvent chloroform–ethyl acetate–formic acid (50 : 40 : 10), catechin (hR_F 34) and epicatechin (hR_F 40) are separated, although some decomposition was detected in prolonged experiments. By using cellulose F with the solvent, 1-butanol–acetic acid–water (40 : 10 : 50, upper phase taken), based on earlier work, an improved separation was obtained (catechin, hR_F 64 and epicatechin 78) and decomposition avoided. Nevertheless, degradation can be avoided on silica gel by prompt development after spot application. The pure standards catechin, epicatechin and 4-hydroxybenzoic acid were used in aliquots of 1 μL alongside 1 μL applications of the crude extract of cube gambir which was first dissolved in methanol–water (1 : 1) containing 1% acetic acid and filtered. After development to 10 cm, and evaporation of the solvent, bands were detected by UV irradiation at 277 nm and measured with a Shimadzu high speed TLC scanner CS-920.

From linear calibration plots of area/concentration and six determinations based on the integrations of 18 spots, cube gambir was found from the TLC method to contain 48.21 ± 1.6% catechin compared with 50.59 ± 0.58% by HPLC. The difference of approximately 2.4% was attributed to incomplete separation by HPLC and TLC densitometry was concluded to be useful for rapid analysis and for industrial monitoring.

Table 11 Comparison of percentage compositional analyses of natural cashew nut-shell liquid (*Anacardium occidentale*) by different method

Method	*Anacardic acid*	*Cardol*	*2-Methylcardol*	*Cardanol*
HPLC	71.65 ± 0.493	22.17 ± 0.398	1.08 ± 0.049	5.13 ± 0.374
GC	71.51 ± 0.230	22.34 ± 0.110	2.75 ± 0.720	3.27 ± 0.110
Densitometry	71.33 ± 1.25	20.28 ± 0.710	3.25 ± 0.720	5.12 ± 0.740

Adapted with permission from Pusey (1969).

Determination of Phenolic Acids in Plant Materials

Phenolic acids occur widely in combined form as esters or glycosides and for their liberation and subsequent quantitative distribution in the plant kingdom, enzymic and basic hydrolysis have been employed. Generally, comparable results are obtained by these two procedures. In the basic method the recovered phenolic acids are percolated in aqueous solution through a polyamide column and then eluted to obtain all the phenolic acids except salicylic acid and gentisic acid which are eluted with methanolic ammonia. The main phenolic acids are recovered with ethyl acetate, concentrated and made up to volume in methanol (10 cm^3, solution A) and the material recovered with methanolic ammonia is similarly treated and made up in methanol (10 cm^3, solution B). The method is an 'off the plate' procedure with quantification by UV spectrophotometry and absorbance at the optimum wavelength compared to the absorbance of pure reference solutions.

Silica gel G was used with solvent 1 (dichloromethane–acetic acid–water: 2 : 1 : 1) for the phenolic acids have one hydroxyl group while solvent 2 (benzene–acetic acid: (45 : 4) was used for phenolic acids with hydroxyl and methoxyl groups and for salicylic acid. Application of known volumes of A and of the standard mixture on one plate was followed by development with solvent 1 and likewise solvent 2 was used with B and the standards on another plate. After drying, the chromatograms bands are detected by examination under UV light and by spraying with methanolic ferric chloride. It was found convenient and satisfactory in practice to cover the portion of the plate devoted to the unknown phenolic acids (A or B) and to spray the identification band with ferric chloride. The phenolic acids in the bands from the unknowns A or B were then marked out, removed by scraping, eluted with methanol and made up to volume for spectral examination under the usual conditions. **Table 13** shows the spectral and hR_F values for a range of phenolic acids.

For quantification, concentration/UV absorbance plots for each phenolic acid were obtained.

Table 12 Percentage composition of mangiferin, colladin and colladonin in Colladonia triquetra by TLC and by HPLC methods

Compound	*TLC*	*HPLC*
Mangiferin	2.96 ± 0.079	3.03 ± 0.131
Colladin	0.105 ± 0.008	0.106 ± 0.004
Colladonin	0.595 ± 0.011	0.604 ± 0.006

Adapted with permission from Tyman (1996b).

Determination of Chloro and Nitrophenols in Water Supplies

Phenolic compounds are widespread in the environment, either from natural sources, or from synthetic operations, and thus may be present in drinking, surface or ground waters. The US Environmental Protection Agency has quoted the compounds 2,4-dimethyl-, 4-chloro-3-methyl-, 2-chloro-, 2,4-dichloro, 2,4,6-trichloro-, 2-nitro, 4-nitro, 2,4-dinitro-, 4,6-dinitro-2-methyl- and pentachlorophenols as priority pollutants. German drinking water regulations call for a limiting value of 0.0005 mg L^{-1}, while in the World Health Organization recommendations for drinking water, maximum standard values are fixed at 0.0001 mg L^{-1} for total chlorophenols. A comprehensive examination of the TLC properties of 39 phenols on aluminium-backed RP-18 F_{254} plates in 12 solvent systems has served as a basis for the detection of significant phenolic pollutants in water supplies.

Water is spiked with the phenols at a concentration of 1 mg L^{-1} (0.0001%), adjusted to pH 2, and then sucked under vacuum through a small pretreated LiChrolut EN column over a period of 3–4 h. The column is dried with a stream of nitrogen for 15–30 min, and the phenols eluted with ethyl acetate (2×0.5 cm^3) and made up to 1 cm^3 with ethyl acetate. Samples of 2 μL are applied on to silica gel 60 F_{254} RP-18 plates (10×20 cm) and developed with dichloromethane-*n*-hexane (50 + 50, w/v) for 20 min. Detection is by UV absorbance at 224 nm with a Camag TLC/HPTLC Scanner II. **Figure 10**

Table 13 UV spectral maxima (methanol), calibration factors (CF*) and hR_F values of phenolic acids (solvents 1 and 2)

Phenolic acid	*Substituent*	*CF*	*Max (nm)*	hR_F	
Hydroxy acid				*Solvent 1*	*Solvent 2*
Salicylic	2-OH	1.74	305	100	67
3-Hydroxybenzoic	3-OH	2.60	298	79	33
4-Hydroxybenzoic	4-OH	0.49	253	86	33
2,3-Dihydroxybenzoic	2,3-OH	2.43	318	89	34
2,4-Dihydroxybenzoic	2,4-OH	1.34	295	78	30
Gentisic	2,5-OH	1.78	330	63	25
2,6-Dihydroxybenzoic	2,6-OH	2.47	317	33	14
Protocatechuic	3,4-OH	0.82	258	44	16
3,5-Dihydroxybenzoic	3,5-OH	2.88	310	20	8
Gallic	3,4,5-OH	0.89	271	11	6
Phloroglucine	2,4,6-OH	0.61	261	10	5
Vanillic	3-OMe, 4-OH	0.81	258	100	58
Syringic	3,5-OMe, 4-OH	0.88	272	100	44
2-Hydroxycinnamic	2-OH	0.98	325	95	34
3-Hydroxycinnamic	3-OH	0.44	276	89	33
Caffeic	3,4-OH	0.56	325	51	14
Ferulic	3-OMe, 4-OH	0.58	320	100	54
Isoferulic	3-OH, 4-OMe	0.64	322	100	49
Sinapic	3,5-OMe, 4-OH	0.61	322	100	39

CF*; concentration (mg 100 cm^{-3}) corresponding to an absorbance of 0.5 g cm^{-2}. Adapted with permission from Vanhaelen *et al.* (1984).

shows the chromatogram obtained with a mixture of five phenols (hR_F values given in brackets), 4-nitrophenol (4.7), 4-chloro-3-methylphenol (16.8) 2,4-dimethylphenol (21.9), 2,4-dinitrophenol (27.1) and 4-nitrophenol (60.3). Their visual and spectral detection limits and recoveries from the column extraction process are shown in **Table 14** along with some values of other phenols which have been similarly processed.

Derivatization of phenols has also been employed to obtain the enhanced absorbance resulting from the formation of coloured derivatives, although the resolution of the resultant compounds may then be diminished.

Conclusions

Although the TLC separation of phenols is well established for qualitative analysis, it is clear that excellent quantitative analysis can be achieved with the vast range of solvent systems, commercial plates and detection equipment currently available. HPTLC and

Table 14 Detection limits and percentage recovery of some pollutant phenols

Compound	*Detection limit (ng)*		*Wavelength (nm)*	*Recovery (%)*
	Visual	*Spectral*		
2-Nitrophenol	100	20	278	86
4-Nitrophenol	100	20	281	97
2,4-Dinitrophenol	100	20	267	99
2-Chlorophenol	1000	100	220	
2,4-Dichlorophenol	400	100	204	97
2,4,6-Trichlorophenol	400	100	207	
Pentachlorophenol	200	20	216	81
4-Chloro-3-methylphenol	400	100	200	97
4-Chloro-2-methylphenol	100	20	224	89
2,4-Dimethylphenol	400	100	223	73
4,6-Dinitro-2-methylphenol	100	20	276	
Phenol	400	200	274	95

Adapted with permission from Bund *et al.* (1995).

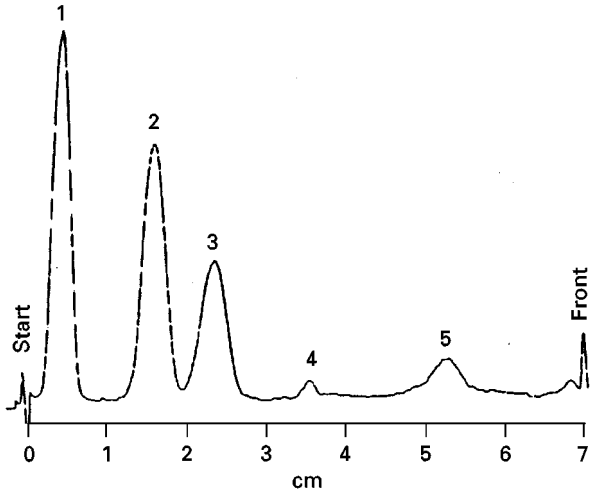


Figure 10 Separation of five phenols (extracted from spiked water) on silica gel RP-18 with UV detection. (Reproduced with permission from Bund *et al.*, 1995.)

RP approaches will extend the range of separative possibilities. Layers impregnated with silver and with other inorganic ions afford the opportunity for selective separations of unsaturated constituents of alkenylphenols and *cis*/*trans* isomers and of benzenoid compounds respectively. Combination of TLC with other chromatographic and/or spectroscopic techniques 'on' or 'off the plate' is likely to offer an economic approach to the analysis of complex mixtures of synthetic and natural phenols.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Mass Spectrometry; Spray Reagents. **III/Impregnation Techniques: Thin-Layer (Planar) Chromatography. Phenols:** Gas Chromatography; Liquid Chromatography; Solid-Phase Extraction. **Silver Ion:** Thin-Layer (Planar) Chromatography.

Further Reading

Baranowska I and Skotniczna A (1994) Behaviour of phenols in normal and reversed-phase thin-layer chromatography. *Chromatographia* 39: 564–568.

Bund O, Fischer W and Hauck HE (1995) The chromatographic properties of 39 phenolic compounds on a RP-18 stationary phase. *Journal of Planar Chromatography* 8: 300–305.

Hanai T (1982) Phenols and organic acids. In: Zweig G and Sherma J (eds) *Handbook of Chromatography*, pp. 127–158. Tables TLC-1–TLC-15. Boca Raton, FL, USA: CRC Press.

Lepri L, Desideri PG and Heimler D (1982) High-performance thin-layer chromatography of chloro-, bromo- and alkylphenols on ready-for-use plates of silanized silica gel alone and impregnated with anionic detergents. *Journal of Chromatography* 248: 308–311.

Petrović M, Kaštelan-Macan M and Horvat AJM (1992) Thin-layer chromatographic behaviour of substituted phenolic compounds on silica gel layers impregnated with Al(III) and Cu(II). *Journal of Chromatography* 607: 163–167.

Petrowitz H-J (1969) Synthetic organic products. In: Stahl E (ed.), *Thin-layer Chromatography*, pp. 657–685. London: George Allen and Unwin.

Pusey DFG (1969) Thin-layer chromatography and the organic chemist. *Chemistry in Britain* 5: 408–412.

Schmidtlein H and Herrman K (1975) Quantitative analysis for phenolic acids by thin-layer chromatography. *Journal of Chromatography* 115: 123–128.

Simova M, Tomov E, Pangarova T and Pavlova N (1986) Determination of phenolic compounds in *Colladonia triquetra* L. *Journal of Chromatography* 351: 379–382.

Smolarz HD and Waksmundzka-Hajnos M (1993) Two-dimensional TLC of phenolic acids on cellulose. *Journal of Planar Chromatography* 6: 278–281.

Tyman JHP (1978) *Journal of Chromatography* 166: 159–182.

Tyman JHP (1987) Analysis of long chain phenols. In: Treiber LR (ed.) *Quantitative Thin-layer Chromatography and its Industrial Applications*, ch. 5, pp. 125–161. New York: Marcel Dekker.

Tyman JHP (1996a) Phenols, aromatic carboxylic acids and indoles. In: Sherma J and Fried BJ (eds) *Handbook of Thin-layer Chromatography*, 2nd edn, ch. 25, pp. 877–905 (Tables 1–19). New York: Marcel Dekker.

Tyman JHP and Morris LJ (1967) The composition of cashew nut-shell liquid (CNSL) and the detection of a novel phenolic ingredient. *Journal of Chromatography* 27: 287–288.

Vanhaelen M, Vanhaelen-Fastré TR, Niebes P and Jans MJ (1984) Thin-layer chromatography-densitometry and high performance liquid chromatography of catechin in cube gambir. *Chromatography* 294: 476–479.