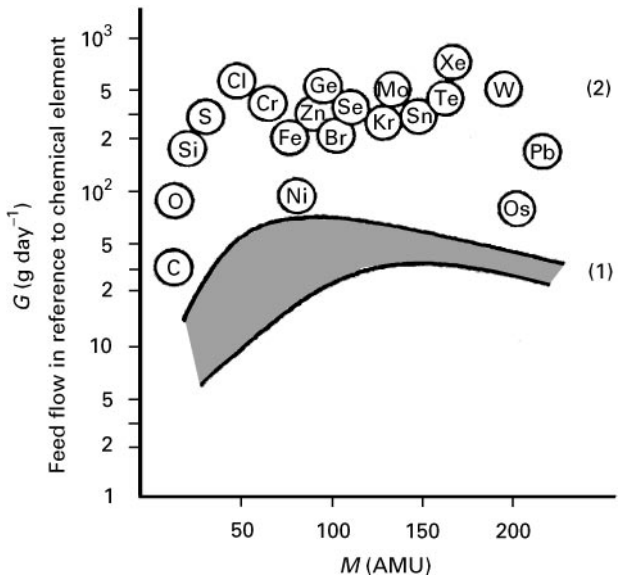


Figure 3 Illustration to compare the separation of nonuranium isotopes by electromagentic separation (1) and by gas centrifuge (2). Production, G (g day^{-1}: feed flow into the installation), versus molecular mass, M, of the separating chemical element. Reproduced with permission from Goldstein *et al.* (1993).

gas impurities. An extremely important benefit of gas centrifuges is the opening of an era of economical large scale production of many stable isotopes that have applications in medicine, industry and fundamental science. For example, experiments on neutrino physics with large detectors that require tens of kg of enriched isotope have been made possible by the cost reductions achieved through the large scale production by gas centrifugation.

Future

The gas centrifuge is now a mature technology, but nevertheless the development potential has not been exhausted. The separation efficiency of existing gas centrifuges for separation of uranium and nonuranium isotopes can be further improved. Additionally, the technology can be applied in the near future to the re-enrichment of uranium from spent nuclear fuel as well as to enriching tails (depleted uranium of separating plants) to the natural isotopic concentration or higher. In yet another area, experiments have been performed which show promising results for the application of gas centrifuge technology to purification of process gas from aerosol particles that can be used, for example, in the semiconductor industry.

See Colour Plate 102.

Further Reading

Avery DG and Davies E (1973) *Uranium Enrichment by Gas Centrifuge*. London: Mills & Boon.

Beams JW and Haynes FB (1936) The separation of isotopes by centrifuging. *Physical Review* 50: 491.

Benedict M, Pigford LH and Levi HW (1981) *Nuclear Chemical Engineering*. New York: McGraw Hill.

Cohen K (ed.) (1951) *The Theory of Isotope Separation as Applied to the Large-scale Production of U^{235}*. New York: McGraw-Hill.

Goldstein S, Louvet P and Soulié E (eds) (1993) *Les Isotopes Stables Application–Production*. Paris: Centre d'Études de Saclay.

Lindemann FA and Aston FW (1919) The possibility of separating isotopes. *Philosophical Magazine* 37: 523.

London H (ed.) (1961) *Separation of Isotopes*. London: George Newnes.

Olander DR (1972) Technical basis of the gas centrifuge. *Advances in Nuclear Science and Technology* 6: 105.

Villani S (ed.) (1979) *Topics in Applied Physics*: *Uranium Enrichment*. New York: Springer-Verlag.

Whitley S (1984) Review of the gas centrifuge until 1962. Parts 1 and 2. *Reviews of Modern Physics* 56: 43.

Liquid Chromatography

L. Lešetický, Charles University, Prague, Czech Republic

Introduction

The application of isotopes in science and especially in analytical chemistry is based on two rather contradictory assumptions. Tracer experiments require that the isotopically modified and unmodified compounds behave in a very similar manner. A study of isotope effects must recognize, measure and interpret minute differences in the chemical and physical properties of compounds (isotopomers) that differ in their isotopic composition. These differences lead to different behaviour of isotopomers in all chromatographic processes, as has been demonstrated experimentally.

As early as 1938, Urey and coworkers published a paper on enrichment of ^{6}Li by ion exchange

chromatography and work in this field has been continuing to date. Gas–solid chromatography has been used for separation of the hydrogen isotopes, noble gases and some other gaseous elements and simple compounds. Low-molecular-mass volatile compounds labelled mainly with hydrogen isotopes have been separated by gas-liquid chromatography. During the late 1950s and the 1960s, papers were published concerning small but observable isotopic fractionation with liquid chromatography of isotopically labelled organic compounds. Over the last 20 years during which liquid chromatography (LC) has become a common analytical technique, many papers have been published dealing with separation of labelled compounds from their unlabelled counterparts. A review of these works, with a possible explanation of the mechanisms of the separation process, is presented.

Isotope Effects

Chromatography can be considered as a process in which the compounds to be separated interact with a stationary and a mobile phase. These mutual interactions differ in magnitude even for different isotopes of the same element. The magnitude of the interaction energy for an isotopic pair depends on many parameters that are discussed below.

It is possible to draw the following general conclusions from the experimental data on isotope effects on the physical properties of molecules.

1. The C–^{2}H bond is shorter than the C–^{1}H bond (e.g. in ethane the C–H bond length is 111.2 pm; in hexadeuteroethane the C–^{2}H bond length is 110.7 pm) and exhibits a higher electron density than the ^{1}H bond. The deuterium atom appears to be smaller than the hydrogen atom. There are small, but measurable differences in the dipole moments, e.g. $\mu(CH_3–^2H) = 3.7 \times 10^{-32}$ C m, $\mu(^1HCl)–\mu(^2HCl) = 1.7 \times 10^{-32}$ C m.
2. The C–^{2}H bond has a polarizability lower than that of the C–^{1}H bond.
3. The C–^{2}H stretching vibrational frequency (around 2200 cm^{-1}) is lower than the corresponding C–^{1}H frequency (around 3000 cm^{-1}).
4. Heavier isotopes have smaller atomic volumes; deuterated compounds have smaller molar volumes than the corresponding unlabelled compounds. The molar volume differences between, e.g. deuterated and unlabelled benzene are due to a molecular size effect caused by differences in the zero-point intermolecular motion of the molecules.
5. Deuterium (^{2}H) is more electropositive than protium (^{1}H) and thus some isotope effects can be discussed in terms of inductive effects. This has been clearly demonstrated in the measurement of the secondary isotope effect on ionization equilibria of some deuterated carboxylic acids and protonated amines, as shown in **Table 1**.
6. Deuterated compounds are less lipophilic than the corresponding unlabelled compounds

Table 1 Isotope effect on dissociation constants[a]

Compound[b]	$log(K_H/K_D)$
^{2}HCOOH	0.035 ± 0.002
C^2H_3COOH	0.014 ± 0.002
$(C^2H_3)_3CCOOH$	0.018 ± 0.001
$C_6{}^2H_5COOH$	0.010 ± 0.002
(2,6-$^2H_2$)benzoic acid	0.003 ± 0.001
$C^2H_3\overset{+}{N}H_3$	0.051
$(C^2H_3)_2\overset{+}{N}H_2$	0.117
$(C^2H_3)_3\overset{+}{H}N$	0.207

[a] Willi (1983), pp. 58–61.
[b] Only the isotope-modified compound is specified in the table.

All these experimentally demonstrated phenomena may influence chromatographic separation processes. Isotope effects on the chromatographic behaviour of compounds labelled with isotopes of heavier elements (carbon, nitrogen, oxygen, etc.) are so small that they can only be detected for simple compounds of low relative molecular mass. The following discussion is limited to deuterated and tritiated compounds and only a few examples are given of separations of heavier isotopes.

It should be pointed out that discussion of isotope effects in terms of inductive or other electronic effects is sometimes helpful but represents a gross simplification. A more exact description of the origin of the isotope effects uses the zero-point energy and the vibrational frequencies of the molecules in question.

Separation Processes

Two basic types of separation process can be distinguished. In systems with a polar stationary phase and nonpolar mobile phase (classical adsorption chromatography, normal-phase LC and chemically bonded polar stationary phase LC) the 'ordinary' (unlabelled) compounds are usually eluted first, i.e. the separation factor is less than unity ($\alpha = k_1/k_2 < 1$; subscripts 1, 2, 3 refer to protium, deuterium and tritium (^{3}H), respectively). As mentioned above, deuterated or tritiated compounds are more polar and thus are more strongly bound to polar stationary phases.

Table 2 LC separation factors of some hydrocarbons versus their perdeuterated analogues

Compound	$\alpha = k_1/k_2$	*Conditions*[a,b]
$[^2H_{14}]$Hexane[a]	1.036	51
	1.049	40
$[^2H_{18}]$Octane[a]	1.054	51
$[^2H_{12}]$Cyclohexane[a]	1.044	33
$[^2H_6]$Benzene[a]	1.043	23
	1.048	18
$[^2H_6]$Benzene[b]	1.049	30
$[^2H_8]$Toluene[a]	1.046	33
	1.057	23
$[^2H_8]$Toluene[b]	1.052	40
$[^2H_{10}]$Phenanthrene[b]	1.067	70
$[^2H_{14}]$Durene[b]	1.071	70
$[^2H_{10}]$Biphenyl[b]	1.062	55

[a] μ-Bondapak C_{18} column; methanol in water (mol%); Tanaka (1977).
[b] Ultrasphere C_{18} column; acetonitrile in water (v/v); Baweja (1987).

Partition chromatography and reversed-phase LC usually lead to the opposite result, i.e. a heavier isotopomer is eluted first ($\alpha > 1$). It has been suggested that a major contribution to the isotope effect is then a hydrophobic interaction. The fact that the separation factors are higher (see **Table 2**) when the mobile phase contains more water demonstrates that they are affected by more restricted motion of the C–H bonds (in solute), caused by a tighter solvation of the C–H bonds within the aqueous mobile phase relative to the hydrophobic stationary phase. On the other hand a less restricted motion of the C–H bonds in the stationary phase would tend to favour protium over deuterium, and this could contribute to the observed isotope effect.

Another important factor is the position of the label in a molecule. As these effects are essentially primary isotope effects, the best situation occurs when an isotopic atom is pushed out from the rest of a molecule, so that it is readily accessible for interaction. When the atom in question is in the shadow of a bulky group (e.g. *ortho* hydrogen in benzoic acid) or in an inconvenient configuration, hydrophobic interactions and adsorption can not take place or are greatly suppressed and no separation is attained. The other contributions, such as differences in solubility, molar volume and adsorption on the residual nonderivatized sites on the silica particles, seem to be of a far less importance for the magnitude of the isotope effect.

The above discussion deals with a direct interaction of an isotopic atom or bond with the stationary and mobile phases (primary isotope effect). However, there also exist chromatographic separations based on secondary isotope effects. In molecules deuterated or tritiated in the position adjacent to an ionizable functional group, the magnitude of the isotope effect is often greatly enhanced. As follows from Table 1 α-deuterated amines are stronger bases than unlabelled amines, and α-deuterated acids are weaker acids than their protiated counterparts. In an elution system with a pH value near the pK_a values of the deuterated/unlabelled amine pair, this isotope-induced base strengthening would alter the ratio of protonated and unprotonated forms, leading to differences in the chromatographic mobility. However, at a pH much higher or much lower than the pK_a value where both labelled and unlabelled amines are completely protonated or unprotonated, the pK_a effect of isotopic substitution on the chromatographic separation is greatly suppressed. A similar effect was observed for labelled carboxylic acids.

Survey of Separations

Hydrocarbons and their Simple Derivatives

A separation of perdeuterated aliphatic and aromatic hydrocarbons has been performed on reversed-phase columns with similar results (**Figure 1**). A higher content of water in the mobile phase leads to higher separation factors (Table 2).

Chromatography of various isotopomers of benzoic acid shows a moderate effect of the number of deuterium atoms on the separation factor; however, an important effect is exerted by the position of the label (**Table 3**). Isotopomers containing deuterium in the *ortho* positions display lower isotopic separation than isotopomers with deuterium in

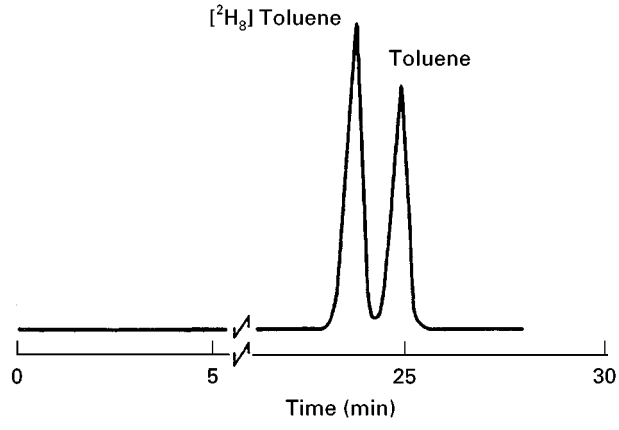


Figure 1 Reversed-phase LC separation of $[^2H_8]$toluene and unlabelled toluene. Mobile phase: water–acetonitrile 60 : 40 (v/v). (From Baweja, 1987).

Table 3 LC separations factors of unlabelled versus deuterated carboxylic acids

Labelled compound	$\alpha = k_1/k_2$
[$^2H_{31}$]Palmitic acid	1.076[a,b]
[$^2H_{23}$]Lauric acid	1.066[a,c]
[$^2H_5$]Benzoic acid	1.040[a,d]
	1.038[e]
[3,4,5-$^2H_3$]Benzoic acid	1.029[e]
[2,3,5-$^2H_3$]Benzoic acid	1.023[e]
[3,4-$^2H_2$]Benzoic acid	1.019[e]
[3,5-$^3H_2$]Benzoic acid	1.019[e]
[2,4-$^2H_2$]Benzoic acid	1.013[e]
[2,5-$^2H_2$]Benzoic acid	1.010[e]
[2,6-$^2H_2$]Benzoic acid	< 1.010[e]

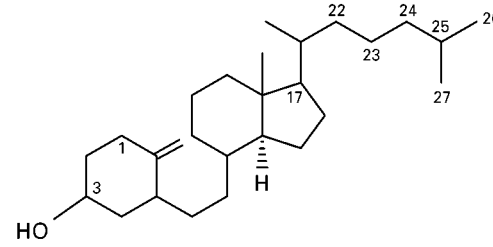
[a] μ-Bondapak C_{18} column; Tanaka *et al.* (1977).
[b] 64 mol%; CH_3OH–H_2O.
[c] 51 mol%; CH_3OH–H_2O.
[d] H_2O, pH 2.51.
[e] Hypersil C_{18} column; CH_3OH–H_2O (3 : 7, v/v) + 1% HCOOH, Lockley *et al.* (1989).

the *meta* or *para* positions. The isotope effects associated with the *ortho* positions are minimal, probably because interactions between the *ortho* C–^{1}H or C–^{2}H bonds and the stationary phase are minimized by steric shielding of the carboxyl group. [9, 10, 12, 13-^{3}H]Linoleic acid methyl ester and [9, 10, 12, 13-^{3}H]oleic acid methyl ester were chromatographed on silica impregnated with silver nitrate, attaining partial separation from the appropriate [1-^{14}C]methyl esters. The tritium atom bound on the olefinic carbon atoms affects the equilibrium constant of the formation of the silver–olefin complex.

Natural Substances

Tritium-labelled steroids have often been used for the elucidation of biochemical and physiological transformations. During the chromatographic purification of such compounds, isotopic fractionation has often been observed. Good, sometimes baseline, separations of tritiated aldosterone, cortisone, oestrone, testosterone, prednisolone and oestradiol from their unlabelled or ^{14}C-labelled analogues were obtained in reversed-phase LC, tritiated compounds being eluted first. Other techniques such as column partition chromatography and paper chromatography have also been successful.

Vitamin D_3 (cholecalciferol, [**I**]) is hydroxylated in living organisms to the 1,25-dihydroxy- or 24,25-



[**I**]

dihydroxy-derivatives. A significant chromatographic isotope effect was observed with both the derivatives labelled with tritium in positions 26 and 27, whereas labelling in positions 23 and 24 causes a substantially smaller effect. A similar behaviour was found in chromatography of the corresponding trimethylsilyl ethers (**Table 4**). As indicated in the table, the heavier isotopomers were eluted first on the reversed phase, and later than lighter isotopomers on the normal phase.

Catalytic hydrogenation with protium, deuterium and tritium of echinocandin B, a macrocyclic peptide

Table 4 LC separation factors of vitamin D_3 metabolites versus their tritiated counterparts

Vitamin D_3 metabolite	$\alpha = k_1/k_3$	*Eluent (v/v)*
1,25-Dihydroxy-[23,24-^{3}H]-TMS[a]	0.977[b,c]	Hexane–CH_2Cl_2 (85 : 15)
	0.989[b,d]	Hexane–CH_2Cl_2–CH_3CN (90 : 10 : 0.035)
1,25-Dihydroxy-[23,24-^{3}H]	0.991[b,d]	Hexane–2-propanol
25(R),26-dihydroxy-[23,24-^{3}H]-TMS[a]	0.965[b,d]	Hexane–CH_2Cl_2 (85 : 15)
1,25-Dihydroxy-[26,27-$^3H_6$]	0.983[e,f]	Hexane–ethanol (94 : 6)
24,25-Dihydroxy-[26,27-$^3H_6$]	0.965[e,f]	
25,26-Dihydroxy-[23,24-^{3}H]	1.008[e,g]	Methanol–water (3 : 1)
1,25-Dihydroxy-[26,27-$^3H_6$]	1.023[e,g]	Methanol–water (1 : 1)

[a] TMS = trimethylsilyl derivative.
[b] Halloran *et al.* (1984).
[c] Zorbax-sil column.
[d] μ-Porasil column.
[e] Worth and Retallack (1988).
[f] Econosphere silica column.
[g] Radial-pak column.

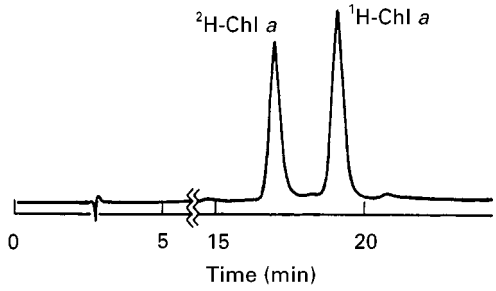
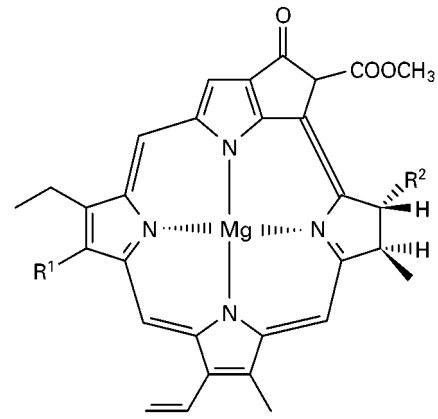
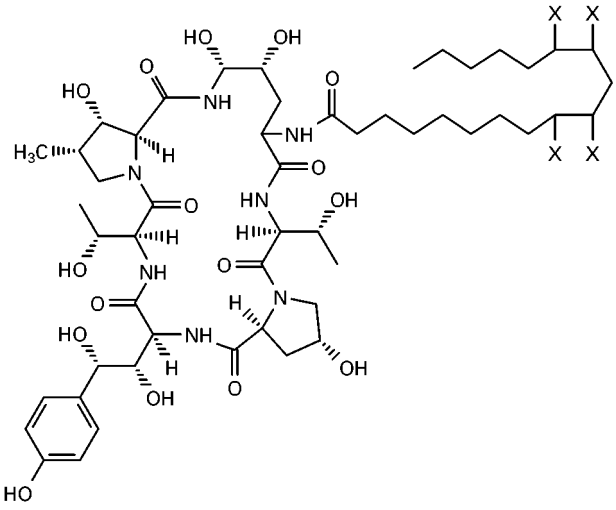


Figure 2 Reversed-phase separation of a deuterated chlorophyll *a* and undeuterated chlorophyll *a* on a 25 cm × 4.6 mm i.d. C_{18}-Ultrasphere ODS column. Mobile phase: water–methanol–acetonitrile–tetrahydrofuran 5 : 28 : 38 : 23 (v/v/v/v). UV detector 663 nm. (From Baweja 1986.)



[III]



[II] X=^{1}H, ^{2}H or ^{3}H

possessing antibiotic and antifungal properties, leads to the corresponding tetrahydroderivatives [**II**]. The isotope effect on the reversed-phase LC mobility was surprisingly large: $\alpha_2 = k_1/k_2 = 1.0190$ for the deuterated compound; $\alpha_3 = k_1/k_3 = 1.0233$ for the tritiated compound. A Partisil 5 C_{18} column and a mixture of 0.1% phosphoric acid (45%) with $CH_3CN/THF/H_3PO_4$ (90 : 20 : 0.1, v/v/v) (55%) as the eluent were used. Even though the chromatographic conditions described do not allow resolution of labelled and unlabelled species, such a possibility exists, e.g. when a column recycling system is used.

Reversed-phase LC of deuterated chlorophylls [**III**] obtained from green algae *Rhodospirillum rubrum* and *Rhodospirillum spheroides* grown in media containing 50%, 80%, 90% and 99.7% ^2H_2O, exhibited baseline separation from isotopically unmodified chlorophyll. As usual, the labelled species were eluted first and the greater is the percentage of deuterium in the compound the faster it moved along the column (**Figure 2**, **Table 5**).

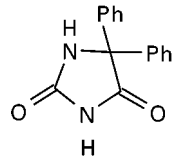
Drugs

[$^2H_{10}$]Diphenylhydantoin (phenytoin [**IV**]) and [$^2H_{10}$]–5H–dibenz[*b*,*f*]azepine–5-carboxamide (carbamazepine [**V**]) were separated from their unlabelled parent compounds by reversed-phase chromatography on a C_{18} column with $H_2O/CH_3CN/THF$ (80 : 16 : 4, v/v/v) as the eluent.

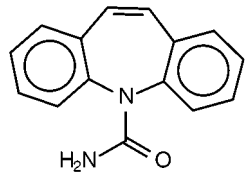
Table 5 LC separation factors of chlorophylls versus deuterated chlorophylls[a]

Labelled compound	$\alpha = k_1/k_2$	*Eluent (A/B)*[b] *(v/v)*
[^{2}H]Chlorophyll *a*	1.132	5 : 95
[^{2}H]Chlorophyll *a'*	1.158	5 : 95
[^{2}H]Chlorophyll *b*	1.156	5 : 95
[^{2}H]Bacteriochlorophyll *a*[c]	1.149	10 : 90
[^{2}H]Bacteriochlorophyll *a*[d]	1.118	7 : 93
[^{2}H]Pyrochlorophyll *a*	1.167	0 : 100

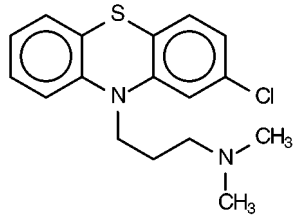
[a]Baweja (1986).
[b]A = H_2O; B = methanol–acetonitrile-THF (30 : 40.5 : 24.5, by vol).
[c]Contains geranylgeranyl side-chain.
[d]Contains phytyl side-chain.



[**IV**]



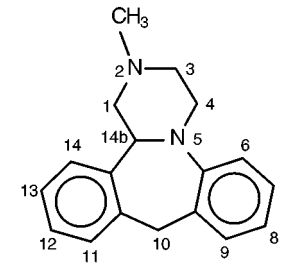
[**V**]



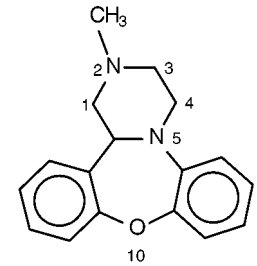
[**VI**]

Baseline separation was attained even when using extracted serum samples. The calculated resolution for the isotopomer pairs was 1.3. (Resolution $R_s = (t_2 - t_1)/[0.5(w_1 + w_2)]$.)

[CH_3–^{3}H]Chlorpromazine [**VI**] and its metabolite [CH_3–^{3}H]-7-hydroxychlorpromazine were separated on a chemically-bonded stationary phase (Spherisorb CN) from their unlabelled counterparts. Although the specific activity of tritiated compound was about 40 Ci mmol^{-1} indicating approximately 1.5 tritium atoms per methyl group, the measured separation factors were rather high (**Table 6**). The separation factors depend strongly on the pH of the mobile phase, so that the isotope effect on the basicity of the dimethylamino group is probably significant. The labelled species was eluted later.



[**VII**]



[**VIII**]

An almost complete resolution was attained between unlabelled and di-, tri- or tetradeuterated drugs active on the central nervous system – 1,2,3,4,10,14b-hexahydro-2-methyldibenzo[*c*,*f*]pyrazino[1,2-a]azepine (mianserin [**VII**]) and 1,3,4,14b-tetrahydro-2,7-dimethyl-2H-dibenzo[*b*,*f*] pyrazino[1,2-a]-1,4-oxazepine (Org GC 94 [**VIII**]). [3,3,4,4-$^2H_4$]Org GC 94 was chromatographed on a μ-Porasil column and n-hexane–2-propanol (90 : 10, v/v) to which 4% of ethanol and 0.1% of ammonia solution were added as the mobile phase. Various isotopomers of deuterated mianserin were chromatographed on LiChrosorb Si 60 and Spherisorb C_{18} columns (**Table 7**). The deuterated compounds were always moving more slowly than the unlabelled ones. The greatest isotope effects have been found for compounds labelled on the piperazine ring (with the exception of position 14b) and on the methyl group,

Table 6 LC separation factors of chlorpromazine versus tritiated chlorpromazine[a]

Labelled compound	$\alpha = k_1/k_3$[b]	*pH of eluent*[c]
[CH_3–^{3}H]Chlorpromazine	0.855	7
	0.901	6
	1.00	5
[CH_3–^{3}H]-7-Hydroxychlorpromazine	0.847	7
	0.910	6
	0.952	5

[a] Yeung *et al.* (1984).
[b] Spherisorb CN column.
[c] 10% 0.05 mol L^{-1} sodium acetate buffer in methanol.

Table 7 LC separation factors of mianserin versus isotopomers of deuterated mianserin[a]

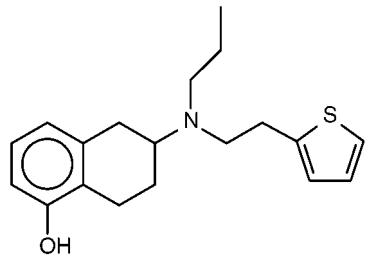
Labelled compound[b]	α_2 (*LiChrosorb*)[c]	α_2 (C_{18})[d]
[3,3,4,4-$^2H_4$]Mianserin	0.893	0.935
[*N*-C^2H_3]Mianserin	0.893	0.935
[3,3-$^2H_2$]Mianserin	0.910	0.935
[1,1-$^2H_2$]Mianserin	0.926	
[4,4-$^2H_2$]Mianserin	0.971	

[a]Kaspersen *et al.* (1984).
[b]For other isotopomers, namely [8-^{2}H], [6,8-$^2H_2$], [10,10-$^2H_2$], [12-^{2}H], [13-^{2}H] and [14b-^{2}H] no fractionation occurred.
[c]n-Hexane–2-propanol (9 : 1, v/v) + 0.1% NH_3, aq.
[d]0.1 mol L^{-1} sodium acetate-acetonitrile (1 : 4, v/v).

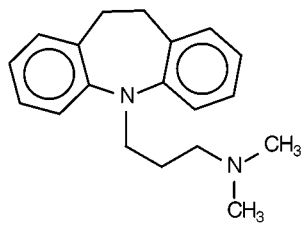
indicating that the basicity change plays the most important role.

2-(*N*-Propyl-*N*-2-thienylethyl)-5-hydroxytetralinamine (N-0437 [**IX**]), an effective drug against Parkinson's disease and glaucoma, can be separated from its counterparts deuterated and tritiated at the propyl group. The corresponding diastereoisomeric glucuronides were prepared by enzymatic synthesis and separated in a reversed-phase LC system (Nova-Pak C_{18}). The separation factors were pH-dependent and relatively large, considering the remote positions of the labels from the nitrogen atom, e.g. $\alpha_2 = 1.033$; $\alpha_3 = 1.037$.

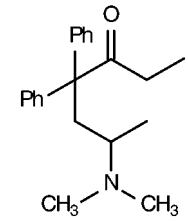
The above-mentioned effect of isotope substitution on the basicity of amines was demonstrated by thin-layer chromatography (TLC) of the popular antidepressant imipramine [**X**]. [$^2H_{10}$]Imipramine labelled in both methyl groups and in aromatic rings and its parent compound were resolved on silica plates using different eluents, in which the presence of ammonia was necessary; e.g. benzene–acetone–NH_3 (300 : 60 : 1, v/v/v); $R_F[^1H] = 0.24$; $R_F[^2H] = 0.18$. Labelling on the benzene ring exerted no effect on the chromatographic mobility.



[**IX**]



[**X**]



[**XI**]

Similar TLC behaviour has been observed with (*R*,*S*)-6-[CH_3-$^2H_6$]dimethylamino-4,4-diphenyl-heptan-3-one.HCl ([$^2H_6$]methadone [**XI**]) (silica gel Merck; benzene–methanol–NH_3; 85 : 15 : 0.65, v/v/v); $R_F[^2H] = 0.58$; $R_F[^1H] = 0.79$. Reversed-phase LC showed baseline resolution, whereas gas–liquid chromatography afforded no separation.

Isotopes of Heavier Elements

Liquid chromatographic separations of the isotopes of elements other than hydrogen have been rather rare. A high-efficiency liquid–liquid chromatography system consisting of porous silica microspheres covered with 25% (w/w) bis(2-ethylhexyl)phosphoric acid in dodecane as the stationary phase and nitric acid as the mobile phase obtained a certain enrichment of heavier isotopes of calcium in the front of the elution curve. Separation factors calculated by Glueckauf (1961) for ^{42}C, ^{45}Ca, ^{48}Ca versus ^{40}Ca were 1.0012–1.0029.

Better resolution was obtained when isotopic pairs of ^{144}Sm–^{154}Sm, ^{140}Ce–^{142}Ce, and ^{151}Eu–^{153}Eu were chromatographed on LiChrosorb 60 with di-2-propylether-THF-65% HNO_3 (100 : 20 : 5, v/v/v). Heavier isotopes were always eluted first, the separation factors being two orders of magnitude higher than in ion exchange chromatography (1.030–1.085).

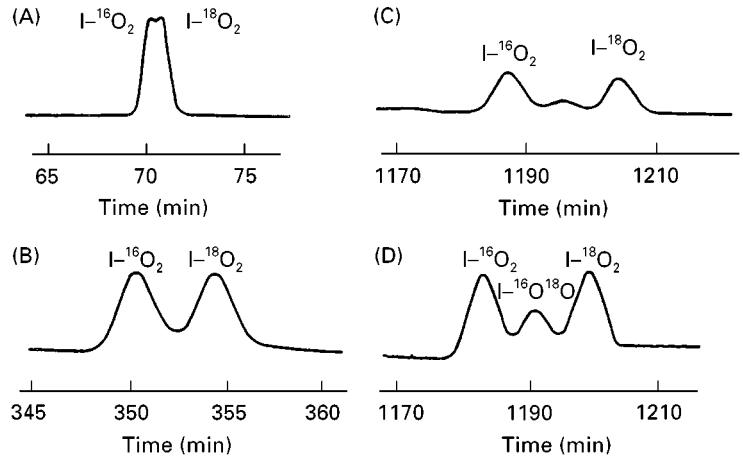


Figure 3 Separation of benzoic acid (I) isotopomers by recycle chromatography. Column: Cosmosil 5-C_{18}-P × 4, 15 cm × 4.6 mm i.d. Mobile phase: methanol-0.05 mol L^{-1} acetate buffer (pH 4.83) 20 : 80 (v/v). Cycles: (A) 1, (B) 5, (C) and (D) 17. (From Tanaka, 1986.)

There are very few references in the literature to liquid chromatographic separations of organic compounds labelled with isotopes heavier than hydrogen. For example, Tanaka *et al.* (1986) demonstrated the oxygen isotope effect on the reversed-phase LC separation of ^{18}O-labelled benzoic acid (**Figure 3**). They demonstrated that successful separation was due to differences in the dissociation constants between the labelled and parent compounds. The isotope effect was pH-dependent and attained a maximal value around pH 5 (**Table 8**). An equation was derived to determine the ^{18}O isotope effect on the dissociation constants from LC data. This isotope effect was calculated to be $^{16}K_a/^{18}K_a = 1.020 \pm 0.002$. A very good, nearly baseline, resolution was attained in a recycle system for the pair benzoic acid – [$^{18}O_2$] benzoic acid after five cycles, and for the mixture benzoic acid – [^{18}O]benzoic acid – [$^{18}O_2$]benzoic acid after 17 cycles. Similar results were obtained in chromatography of [$^{18}O_2$]-4-chlorobenzoic acid and [OH–^{18}O]-4-nitrophenol with their unlabelled counterparts. The ^{18}O–H bond is stronger than the ^{16}O–H bond so that ^{18}O-labelled acids are weaker acids than unlabelled ones. Therefore, a longer retention time in reversed-phase LC was expected and found for the labelled carboxylic acid compared to the unlabelled acid.

The same procedure as above was successfully applied to the separation of aniline and [^{15}N]aniline on the same column with 0.05 mol L^{-1} acetate buffer containing 5% methanol and 0.01% triethylamine as the eluent. At pH 4.24 the separation factor was found to be $\alpha = k_{14}/k_{15} = 1.010$. The complete separation was accomplished after 20 cycles.

See also: **II/Chromatography; Liquid:** Mechanisms: Normal Phase; Mechanisms: Reversed Phases. **III/ Pharmaceuticals:** Thin-Layer (Planar) Chromatography.

Further Reading

Baweja R (1986) HPLC separation of deuterated photosynthetic pigments from their protio analogues. *Journal of Chromatography* 369: 125–131.

Baweja R (1987) Application of reversed-phase HPLC for the separation of deuterium and hydrogen analogues of aromatic hydrocarbons. *Analytica Chimica Acta* 192: 345–348.

Filer CN (1999) Isotopic fractionation of organic compounds in chromatography. *Journal of Labelled Compounds and Radiopharmaceuticals* 42: 169–197.

Glueckauf E (1961) Isotope separation by chromatographic methods. In: London H (ed.) *Separation of Isotopes*, pp. 209–248. London: G. Newnes Ltd.

Table 8 Dependence on eluent pH of LC[a] separation factors of benzoic acid versus [$^{18}O_2$]benzoic acid[b]

	Eluent pH							
	2.44	3.13	4.07	4.40	4.90	5.29	5.86	6.27
α	1.000	1.000	0.995	0.991	0.988	0.988	0.992	0.995

[a]Cosmosil C_{18}-P; methanol-0.05 mol L^{-1} acetate buffer (20 : 80); 30°C.
[b]Tanaka *et al.* (1986).

Halloran BP. Bikle DD and Whitney JD (1984) Separation of isotopically labelled vitamin D metabolites by HPLC. *Journal of Chromatography* 303: 229–233.

Kaspersen FM, van Acquoy J, van de Laar GLM, Wagenaars GN and Funke CW (1984) Deuterium isotope effects in mianserin. *Recueil Travaux Chimique de Pay-Bas* 103: 32–36.

Klein PD (1966) Occurence and significance of isotope fractionation during analytical separation of large molecules. *Advances in Chromatography* 3: 3–65.

Lešetický L (1985) Isotope separation by chromatographic methods [in Czech]. *Radioisotopy* 26: 113–126.

Lockley WJS (1989) Regiochemical differences in the isotopic fractionation of deuterated benzoic acid isotopomers by reversed phase HPLC. *Journal of Chromatography* 483: 413–418.

Simon H and Palm D (1966) Isotope effects in organic chemistry and biochemistry [in German]. *Angewandte Chemie* 78: 993–1007.

Tanaka N, Araki M and Kimata K (1986) Separation of oxygen isotopic compounds by reversed-phase liquid chromatography. *Journal of Chromatography* 352: 307–314.

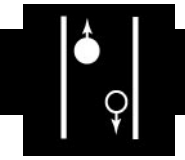
Tanaka N and Thornton ER (1977) Structural and isotopic effects in hydrophobic binding measured by HPLC. A stable and highly precise model for hydrophobic interaction in biomembranes. *Journal of American Chemical Society* 99: 7300–7306.

Willi AV (1983) *Isotope Effects in Chemical Reactions* [in German]. Stuttgart: G. Thieme.

Worth GK and Retallack RW (1988) Tritium isotope effect in high-pressure liquid chromatography. *Analytical Biochemistry* 174: 137–141.

Yeung PKF, Hubbard JW, Baker BW, Looker MR and Midha KK (1984) Isotopic fractionation of *N*-([^{3}H]methyl)chlorpromazine and *N*-([^{3}H]methyl)-7-hydroxychloropromazine by reversed-phase high performance liquid chromatography. *Journal of Chromatography* 303: 412–416.

LEAD AND ZINC ORES: FLOTATION



M. Barbaro, Instituto Trattamento Minerali CNR, Rome, Italy

Outline of the Problem

Today, owing to the limitation of sources and supplies of mineral raw materials and the need to treat ores of increasingly lower grades, as well as those which are fine, complex mineralogically, and refractory, new flotation technology must be developed.

Flotation is in fact the most common process in metallic mineral separation and is the main way for recovering such valuable metals as lead (Pb) and zinc (Zn), or copper (Cu) from ores.

It is known that separation by flotation of useful minerals from gangue in an aqueous pulp happens when particles with polar, hydrophilic or wettable surfaces remain in the liquid phase, whilst particles with apolar hydrophobic or not wettable surfaces adhere to air bubbles. Collectors or depressing/activating reagents modify surface characteristics of minerals thus influencing affinity towards water. Thus, research into new separation technologies is mainly concerned with the search for new flotation reagents.

In fact the value of a flotation concentrate containing a given mineral, from which a desired metal is extracted by a metallurgical process, decreases with an increase in the presence of minerals containing metals other than the one of prime interest. It is thus necessary to design new specific collectors to separate the desired mineral from the gangue. Collectors generally employed in flotation are surfactants that form, for instance, electrostatic bonds with the solids (Leja 1982). Difficulty therefore arises when a particular metallic mineral, such as Pb or Zn mineral, has to be separated from an ore of complex composition or low grade (complex sulfide ores) or when the surface properties of the mineral (oxidized Pb and Zn ores) make the response to flotation extremely poor. To overcome this basic drawback in metal ore flotation, the possibility of using new compounds endowed with a strong affinity for metals themselves has been investigated. The search for new reagents for mineral flotation therefore aims to discover collectors (or depressants) capable of linking more selectively with a given element present in the ore.

The present work deals with the recovery of Pb and Zn by flotation and reviews the main problems faced by research engineers in this field.