Adsorbents for Chromatography

Conquer your toughest chromatography separations

- Alumina
- Silica
- Medium Pressure Liquid Chromatography
- High Performance Liquid Chromatography
- Dry Column Chromatography
- Thin-Layer Chromatography
- Polyamides
- Dehydration of Solvents

MP

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80 Years of Dedication to Chromatography and Filtration for Laboratory and Industrial Applications

In the late 1940s, Woelm Pharma implemented standardization in its production procedures for Aluminas. This resulted in a standardized adsorbent providing a high degree of reproducibility when used in laboratory and plant operations.Woelm Pharma's success with Aluminas encouraged them to carry out similar improvements for other adsorbent products, such as Silica Gels, products for Thin Layer Chromatography (TLC) and Dry Column Chromatography (DCC).

In 1984, ICN Biomedicals acquired the adsorbents division of Woelm Pharma and continued to introduce steady improvements to provide optimum separation products at affordable prices. In 1985, a new manufacturing plant with the latest equipment was constructed at Eschwege, in the heart of Germany.

In 2003, ICN decided to separate their Pharmaceuticals, Research Products and Diagnostics businesses. The Research Products business, including the production and distribution of the Adsorbents and Diagnostics business, was acquired by MP Biomedicals.

Our many years of experience in producing adsorbents for chromatography enables us to offer unique products, such as Super I Aluminas and active Silica Gels. Additionally, we can provide custom-made Aluminas and Silicas to meet the exact needs of our customers. MP Bio will continue to dedicate resources towards the manufacture of high-quality adsorbents to support laboratory and production-scale chromatography applications.

CONTENTS

MP EcoChrom™ Alumina, Activity Super I

Aluminas of activity Super I were originally developed and produced by Woelm. MP EcoChrom™ Alumina Super I are commonly used for purification processes of all types, including sample preparation for pesticides, environmental analysis, solvent purification *(see page 35 for examples)* and chromatography applications requiring high precision and reproducibility.

The sorption behavior of MP EcoChrom™ Alumina Super I is extremely constant for all types of chromatography and different solvent eluents. In a non-polar environment, MP EcoChrom™ Alumina activity Super I shows a sorption capacity approximately twice as high as that of MP EcoChrom™ Alumina Activity I. Their initial activity is kept in a very narrow range and the deactivation behavior is extremely constant.

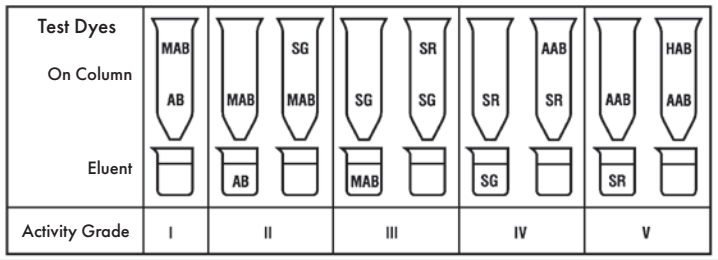
MP EcoChrom™ Alumina Super I are available as basic, neutral, and acid Alumina. The neutral Alumina (MP EcoChrom™ Alumina N - Super I) is nearly free of superficially attached sodium ions. It does not consist of a mixture of acid and basic Alumina, as may occasionally be found in the market from other suppliers.

The high constancy of the initial activity and the deactivation behavior of MP EcoChrom™ Alumina Super I enabled a modification of the original Brockmann and Schodder test, developed in 1941, in such a way that it is now possible to determine the activity of alumina much more precisely. With the modified test, it is possible to fine tune the activity and deactivate MP EcoChrom™ Alumina to precisely determined levels.

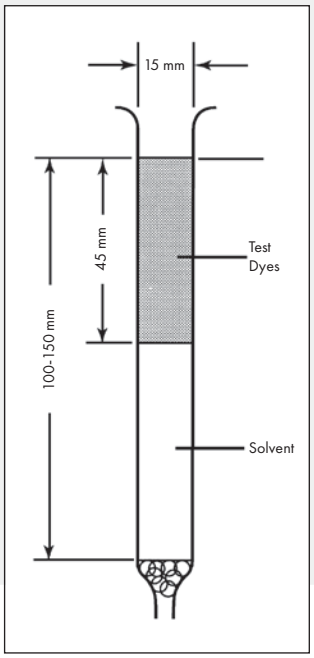
Product Name	pH Value approx.	Loss on Ignition approx. % (1000°C/12h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Alumina A - Super I acid pH, 63-200 µm	4.5	1.3	0.1	0.8	200	500 g	0204592
						1 kg	0204595
						5 kg	0204598
						50 kg	0204601
Alumina B - Super I basic pH, 63-200 µm	10	1	0.1	0.8	200	500 g	0204568
						1 kg	0204571
						5 kg	0204574
						50 kg	0204577
Alumina N - Super I neutral pH, 63-200 µm	7.6	1	0.1	0.8	200	500 g	0204580
						1 kg	0204583
						5 kg	0204586
						50 kg	0204589

Determination of Activity using the Brockmann and Schodder Test

The Brockmann and Schodder Test is a simple and reliable method to determine the activity of adsorbents. A standardized volume of pairs of test dyes dissolved in a standard solvent is applied to a standardized column. After chromatographic development, the activity is revealed by the manner in which the test dyes are separated.



- The following dye mixtures are used:
  - **I** Azobenzene (AB) and p-methoxyazobenzene (MAB)
  - **II** p-methoxyazobenzene (MAB) and Sudan yellow (SG)
  - **III** Sudan yellow (SG) and Sudan red (SR)
  - **IV** Sudan red (SR) and p-aminoazobenzene (AAB)
  - **V** p-aminoazobenzene (AAB) and p-hydroxyazobenzene (HAB)
- 20 mg of each of the two dyes of an individual pair is weighed into 50 mL of the solvent.
- A mixture of one part of pure benzene and four parts of pure petroleum ether (bp 50 – 70°C) (v/v) is used as solvent for the test dyes and as eluent for the chromatographic development.
- 10 mL of the appropriate test dye solution is carefully applied to the top of a 15 mm diameter column, filled to exactly 50 mm in height with the adsorbent to be tested. The column will be eluted with 20 mL of the eluent. The activity grades can be determined according to the diagram below.



Woelm suggested a modification to the Brockmann and Schodder method, using columns of 100 – 150 mm length instead of exactly 50 mm high adsorbent beds. On these columns, elution does not occur. To determine the activity grade, the migration distance of the test dye in front is measured. The activity grade is then assigned by the number of the pair of test dyes, in addition to the distance, in mm from the top of the column to the front of the foremost migrated dye.

In the example, test dye solution #I is utilized and azobenzene migrates 45 mm.
The activity grade of the adsorbent is I/45 mm.

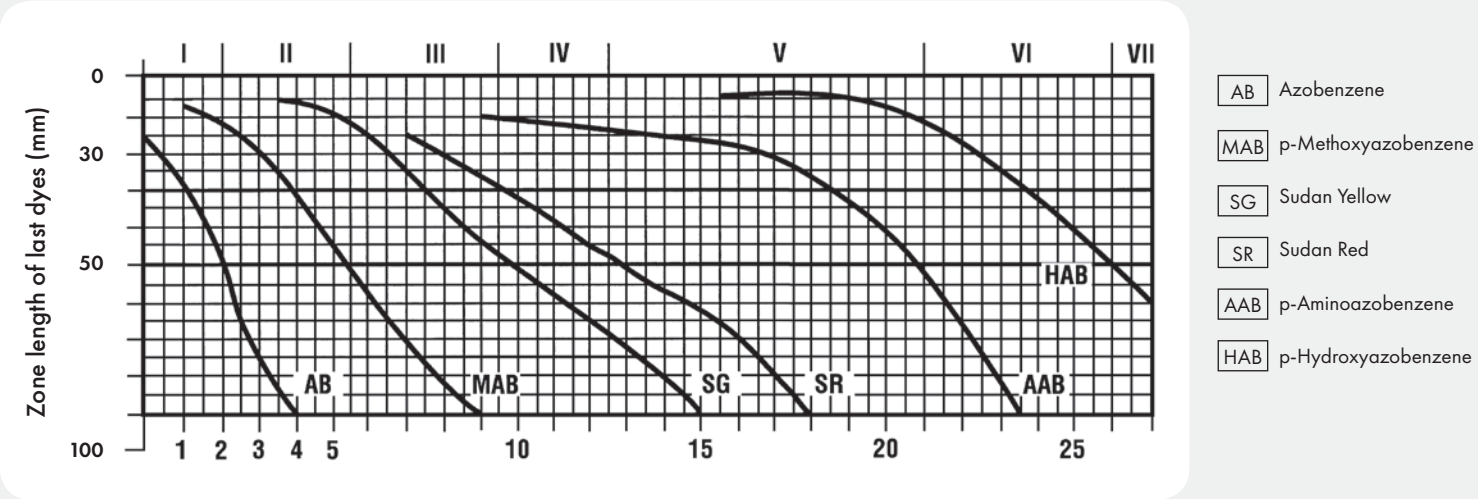
Product Name	Size	Cat. No.
Test dyes for the Determination of Acitivity according to Brockmann and Schodder	1 kit	0209670

Deactivation of MP EcoChrom™ Alumina

MP EcoChrom™ Alumina may be deactivated by the addition of polar media (preferably water), or alcohols such as glycol or glycerol. Monitoring the deactivation with the modified Brockmann and Schodder test allows a very sensitive and controlled deactivation in small increments, as shown in the graph below. The deactivation behavior of MP EcoChrom™ Alumina Super I is standardized to such a high degree that all three surface types of Aluminas (acid, basic, neutral) show an identical initial activity. When deactivated with equal amounts of deactivator, they reach the same lower activity status.

The amount of water required for deactivation can be read off the graph below:

Activity grades acc. to Brockmann



Alternatively, MP EcoChrom™ Alumina Super I may be deactivated according to the classical Brockmann grades, using the deactivation instructions in the following table:

Deactivation of MP EcoChrom™ Alumina, Activity Super I

Activity:	Super I	I	II	III	IV	V	
Alumina A - Super I	0	1	4	7	10	19	% water to be added
Alumina B - Super I	0	1	4	7	10	19	% water to be added
Alumina N - Super I	0	1	4	7	10	19	% water to be added

A known amount of MP EcoChrom™ Alumina is weighed into a vessel and the required amount of the deactivator is added (w/w). The vessel is sealed and shaken until all lumps have dissolved. After cooling to room temperature (at least two hours, preferably overnight), the deactivated Alumina is ready for use. To conserve the activity of the prepared Alumina, the vessel must be kept tightly closed.

MP EcoChrom™ Alumina, Activity I

MP EcoChrom™ Alumina Activity I are good "all-around" adsorbents for laboratory and industrial use. These Alumina are original products, produced to MP's rigid production specifications. They can be adjusted to lower activity by the addition of polar media (preferably water), or higher alcohols such as glycol or glycerol.

For the deactivation of MP EcoChrom™ Alumina Activity I using water, refer to the table below. An activation of MP EcoChrom™ Alumina Activity I to a higher activity by means of standard laboratory equipment is not possible.

Deactivation of MP EcoChrom™ Alumina, Activity I						
Activity:	I	II	III	IV	V	
Alumina A, Act. I	0	3	6	10	15	% water to be added
Alumina B, Act. I	0	3	6	10	15	% water to be added
Alumina N, Act. I	0	3	6	10	15	% water to be added

Product Name	pH Value approx.	Loss on Ignition approx. % (1000°C/12h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Alumina A, Act. I acid pH, 63-200 µm	4.5	1.7	0.1	0.8	150	500 g	0202099
						1 kg	0202102
						5 kg	0202105
						50 kg	0202159
Alumina B, Act. I basic pH, 63-200 µm	10	1.7	0.1	0.8	150	500 g	0202069
						1 kg	0202072
						5 kg	0202075
						50 kg	0202078
Alumina N, Act. I neutral pH, 63-200 µm	7.4	1.7	0.1	0.8	150	500 g	0202084
						1 kg	0202087
						5 kg	0202090
						50 kg	0202135

MP EcoChrom™ Alumina, Activity II - III

MP EcoChrom™ Alumina Activity II - III is an economical adsorbent of medium activity. This Alumina is often used when activated charcoal cannot due to its organic nature, or when the cation exchange properties of basic alumina are desired.

MP EcoChrom™ Alumina Activity II - III is suitable for the development of separation methods and for technical separations where a custom-made adsorbent for special separation is not available.

Product Name	pH Value approx.	Loss on Ignition approx. % (1000°C/12h)	Loss on Drying approx. % (180°C/2h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m²/g	Size	Cat. No.
Alumina, Act. II-III basic pH, 63-200 µm	10	–	–	–	0.8	–	500 g	0204692
							5 kg	0204691
							50 kg	0204694

MP EcoChrom™ Alumina R for Isotope Preparation

MP EcoChrom™ Alumina R is an acid alumina, developed for use in isotope chemistry. Alumina R is a major compound of technetium generators, so-called technetium cows. These are small columns filled with Alumina R, in which the mother nuclide ^{99}Mo (t = 66 h) is retained as the Molybdate MoO_4, while the daughter nuclide ^{99M}Tc (t = 6 h) may be easily removed (milked) as the pertechnetate TcO_4, by elution with physiological salt solution.

MP EcoChrom™ Alumina R may also be used whenever an alumina is required with extremely low pH-value and/or high ion-exchange capacity. An example is the determination of catecholamines according to Anton and Sayre. The authors recommend the preparation of acid alumina by heating basic alumina with hydrochloric acid several times, followed by multiple washes. This time-consuming procedure can be avoided by using MP EcoChrom™ Alumina R.

Product Name	pH Value approx.	Loss on Ignition approx. % (1000°C/12h)	Loss on Drying approx. % (180°C/2h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m²/g	Size	Cat. No.
Alumina R acid pH, 63-200 µm	4.3	–	< 5	–	0.8	–	500 g	0206034
							50 kg	0206031

MP EcoChrom™ Alumina B - Super I for Dioxin Analysis

The most important step in pollution analysis is separation of pollutants from the sample matrix. However, it is impossible to determine the efficiency of the extraction methods. Even if added standards or markers are 100% recovered, it does not give information about the extraction efficiency of the pollutants from the matrix. Because of this uncertainty, cleanup steps must be extremely reliable to avoid the analysis becoming useless.

As matrix extracts are usually processed by chromatography, the quality of the adsorbent used is essential. The highly standardized MP EcoChrom™ Alumina Activity Super I are best suited for this purpose.

To meet the high recovery level required for the analysis of polychlorinated dibenzodioxins and dibenzofurans according to VDI-Regulation 3499, part I, MP Bio developed MP EcoChrom™ Alumina B - Super I for Dioxin Analysis.

With this Alumina, MP Bio has given environmental analysts a powerful tool to improve their work. MP EcoChrom™ Alumina B - Super I for Dioxin Analysis may also be used for all chromatographic processes requiring the highest activity and/or reproducibility.

For dioxin analysis, we recommend the literature cited below, describing the isolation of the most toxic 2,3,7,8-TCDD from other congeneric polychlorinated dibenzodioxins and dibenzofurans using chromatography with MP EcoChrom™ Alumina.

Hagenmaier, H. et al. *Fresenius Z Anal Chem.* **1986**, *323*, 24-28

Product Name	pH Value approx.	Loss on Ignition approx. % (1000°C/12h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m²/g	Size	Cat. No.
Alumina B - Super I for Dioxin Analysis basic pH, 63-200 µm	10	0.7	0.1	0.8	200	500 g	0204569

MP EcoChrom™ Aluminum Oxides for Industrial Applications

Product Name	Size	Cat. No.
Aluminum Oxide acid technical A	50 kg	0207895
	100 kg	0207896
Aluminum Oxide basic technical A	50 kg	0202025
	100 kg	0202027
Aluminum Oxide basic technical A-H 32001-	50 kg	0206025
Aluminum Oxide technical -H 31101-	100 kg	0202018
Aluminum Oxide 1-3 mm	5 kg	0202031
	50 kg	0202029
Aluminum Oxide neutral -H 15152-	50 kg	0204655
	100 kg	0204654

Custom variations are available. Contact us for more information.

Applications of Aluminum Oxide (Alumina)

Alkaloids	Isolation from ergot-, opium-, rauwolfia-, and other alkaloids
Antibiotics	Isolation, Purification
Dehydration of organic solvents	
Enzymes	Purification
Essential oils	Removal of terpenes
Fermentation broth	Isolation of active substances
Glycosides	Isolation of digitalis-, strophantus-glycosides, etc.
Hormones	Isolation and purification of synthetic products, of ketosteroids from neutral materials, etc.
Oils	Clarification of fatty oils, separation of fatty oils
Plant Extraction	Isolation of active substances
Purification of peroxides	For analytical and technical purposes
Removal of peroxides	From organic solvents
Removal of pyrogens	From injectable solutions and infusions
Steroids	Isolation, purification
Solvents for optical purposes	Preparation of chromatographically pure solvents from technical grades
Vitamins	Isolation, purification

Al_2O_3 neutral

Elution Chromatographic Separation of Free and Esterified Cholesterols in Organ and Serum Fats and Direct Colorimetric Determination

Schön, H.; Gey, F. *Hoppe-Seyler's Z. physiol. Chem.* **1956**, *303*, 81.

After extracting the lipoids, a certain amount of material is separated on an alumina column by elution chromatography. The fractions containing free and esterified cholesterol are measured colorimetrically by the Tschugaeff reaction.

In order to carry this out, the starting material is extracted for at least one hour with boiling aldehyde-free ethanol. During the one hour, prepare a 12 mm Ø column of Alumina Woelm (now MP Alumina) neutral, which has been deactivated from activity I to activity II by adding 3% of water. In order to separate out up to 20 mg of fat, 10 grams of alumina will be required. Mash the alumina with petrol ether, place the mixture into the column, and add the fat, dissolved in 5 mL petrol ether.

By eluting with 75 mL of petrol ether containing 4% of peroxide-free ether, the cholesterol esters can be extracted; by using 75–100 mL of petrol ether containing 40% of peroxide-free ether, one can obtain all the free cholesterol together with any triglycerides that may be present. To check whether elution is complete, 25 mL of petrol ether/ether mixture (1:1) is poured through the column and the fraction is tested with the Tschugaeff reaction.

If more than half the total fats present consist of triglycerides, it is wiser to elute more slowly (e.g. at 1 mL instead of 3 mL per minute) and reduce the rate of throughput by using a tap at the outlet of the column. The cholesterol ester is eluted with 75–100 mL petrol ether (containing 8% ether), and the free cholesterol with 75–100 mL petrol ether (with 25% ether). This is checked by eluting once more with 25% mL of petrol ether/ether mixture (1:1).

When dealing with fats of unknown constitution it is better to make a preliminary run at a higher rate of throughput.

For the column size indicated above, one can estimate the fats in 0.1–0.5 mL of serum, 5–15 mg of liverfat, or 5–20 mg of epidermal fat.

In every case, the elute is collected in a 100 mL flask, carefully dried and preserved under nitrogen for later colorimetric determination. The color reaction, using zinc chloride in glacial acetic acid with acetyl chloride and subsequent optical measurement, is carried out as described by the authors.

It takes from 3-4 hours to carry out fractions in 10 separate columns and an additional 2 hours to estimate the esters and cholesterol fractions colorimetrically.

Al_2O_3 neutral

A Quantitative Method for the Chromatographic Separation of 17-oxo Steroid Sulfates from 17-oxo Steroid Glucuronides: with Observation on the Behavior of Conjugated Corticosteroids on the Same System

Barlow, J.J.; Kellie, A.E. *Biochem. J.* **1959**, *71*, 86.

The ether-ethanol extract (3:1) from urine is chromatographed on Alumina Woelm (now MP Alumina) neutral, activity I, deactivated with 5% water and washed with absolute ethanol. The elution with water-ethanol (1:1) yields steroid sulfates, pigments, corticosteroid metabolic substances and non-steroids. The conjugated steroids are then eluted with acetate buffer along with 95–100% of the glucuronides of the corticosteroid metabolic substances. The composition of the eluates is determined by acetylation of the glucuronic acid residue. The glucuronides are thus made insoluble and can be separated from the water-soluble steroid sulfates. This allows a rapid determination of the 17-oxo steroid sulfate/glucuronide ratio.

Determination of Primary Biogenic Amines in Plants, Especially in Ergot (*Claviceps purpurea* Tul.)

Biogenic Amines (Plants)

Al_2O_3 basic

Kordts, D.; Voigt, R.; Weiss, F. *Pharmazie.* **1960**, *15*, 586.

The determination of primary biogenic amines is important for the clarification of plant physiological and pharmaceutical problems. Plants with a high oil content and a great number of amines in different micro quantities are particularly problematic. It has proven useful to convert the bases to their 2,4-dinitrophenyl derivatives and pre-separate them by chromatography on Alumina Woelm (now MP Alumina) basic, activity IV. After fraction elution, the DNP-amines can be separated by paper chromatography and determined with ninhydrin. Experiments with mixtures of the amines in known concentrations confirmed the results obtained with plant material. With secale cornuture the following fractions were obtained: (I) DNP-monoalkylamines and DNP-ammonia, (II) DNP-ammonia, (III) bis-DNP-histamine and DNP-ethanolamine.

Al_2O_3 neutral

Isolation and Structure of the Antibiotics Phomine and 5-Dehydrophomine

Rothweiler, W.; Tamm, Ch. *Helv. Chim. Acta.* **1970**, *53*, 696.

From cultures of a Phoma species (*Fungi imperfecti*) two new metabolites, phomine and 5-dehydrophomine, could be isolated, revealing their structures.

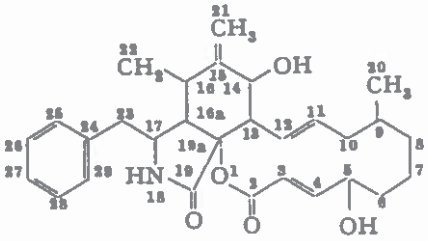
In order to obtain these two metabolites, the cultivation of phoma (strain S 298) was carried out under light exclusion in cultures (30 liters of medium solution in 300 Erlenmeyer flasks at 18–20 °C and pH 4.8). After filtering off the culture broth from the mycel, the latter was washed thoroughly with water. 10 L each of the culture filtrate were stirred out with ethyl acetate and the solvent was washed with the washwater of the mycel. The extract was dried over calcium chloride, filtered and then concentrated in vacuo. By this method, 4-6 g of dark brown raw extract could be obtained. After a few days, this raw extract showed crystallization rudiments.

The raw extract was dissolved in methylene chloride/methanol (9:1) and filtered over two to three times the amount of Aluminum Oxide Woelm (now MP Aluminum Oxide) neutral, act. II. By fractional crystallization from acetone/ether, most of the phomine could be obtained from the purified raw extract. The residues of the mother liquid were chromatographed over the 40-fold quantity of the same alumina. The elution of phomine was carried out with methylene chloride/methanol (998:2). The elution of the only somewhat faster running 5-dehydrophomine was performed with methylene chloride.

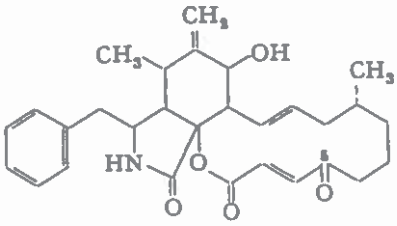
The yield of phomine was 80–120 mg per 1 L culture broth; the yield of 5-dehydrophomine was 40–80 mg per 1 L culture broth. Phomine forms colorless prismatic needles with a melting point of 218–220 °C, and 5-dehydrophomine forms colorless needles with a melting point of 185–187 °C.

These two metabolites are the first representatives of a novel type of macrolide antibiotics. The 14-membered lactone ring is joined to a highly substituted octahydro-isoindole system. The metabolites show cytostatic activity in vitro.

The following sum formulas were found: $C_{29}H_{37}NO$ for phomine, $C_{29}H_{35}NO$ for 5-dehydrophomine. Based on further tests the following structure formulas were found:



Phomine



5-Dehydrophomine

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MP EcoChrom™ Silica Gels

Silica gels are the most widely used oxidic sorbents for adsorption chromatography and partition chromatography. They are used in a variety of applications, from analytical micro-bore columns to the largest columns for industrial use.

The physical characteristics of silica gels (e.g. specific surface, pore size and volume, and particle size) can vary over a wide range. This makes it easy to adapt the silica gel to a specific separation situation. Silica gels have only minor catalytic activity, so the formation of artifacts and the degradation of compounds to be separated will be minimal. Thus, partition chromatography with silica gel is well suited for the purification of compounds sensitive to degradation and for the separation of unknown mixtures.

Silica gels of 60 Å pore size are most commonly used. They are suitable for adsorption chromatography and partition chromatography, of natural and synthetic products of moderate molecular weight. MP Bio offers a wide range of 60 Å Silica gels (MP EcoChrom™ Silica) with a specific surface of about 500 to 600 m^2/g for use in all areas of partition chromatography, and activated (MP EcoChrom™ Silica, active) for adsorption chromatography.

MP EcoChrom™ Silica

All MP EcoChrom™ Silicas are made from the same raw material. Their sieve cuts are optimized according to the sieve range of the German Industrial Standard (DIN). MP EcoChrom™ Silica are free of particles outside the standard range. Their irregular shape permits the easy packing of columns of different sizes, and the optimization of column permeability and plate number. They allow easy switching from one sieve cut to another, offering efficient means for changing chromatographic methods.

Product Name	pH Value approx.	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Silica 0-63 µm, 60 Å	7	0.2	0.4	500-600*	500 g	0204666
					25 kg	0204668
Silica 18-32 µm, 60 Å	7	0.2	0.4	500-600*	10 g	0202745
					100 g	0202757
					500 g	0202753
					1 kg	0202754
					2.5 kg	0202755
					25 kg	0202830
Silica 32-63 µm, 60 Å	7	0.2	0.4	500-600*	500 g	0202824
					1 kg	0202825
					2.5 kg	0202827
					15 kg	0202756
					25 kg	0202826

*when properly activated

MP EcoChrom™ Silica - *cont.*

Product Name	pH Value approx.	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Silica 40-63 µm, 60 Å	7	0.2	–	500-600*	500 g	0208120
					1 kg	0208121
					2.5 kg	0208122
					25 kg	0208123
Silica 32-100 µm, 60 Å	7	0.2	0.5	500-600*	500 g	0202758
					25 kg	0202759
Silica 63-100 µm, 60 Å	7	0.2	0.5	500-600*	500 g	0204641
					25 kg	0204660
Silica 63-200 µm, 60 Å	7	0.2	0.5	500-600*	500 g	0204662
					1 kg	0204664
					2.5 kg	0204663
					25 kg	0204667
Silica 100-200 µm, 60 Å	7	0.2	0.5	500-600*	500 g	0202760
					25 kg	0202761
Silica 200-500 µm, 60 Å	7	0.2	0.5	500-600*	500 g	0202811
					25 kg	0202809

*when properly activated

MP EcoChrom™ Silica, active

In addition to the conventional silica, MP Bio produces active silica. These MP EcoChrom™ Silica, active, exhibit a well-defined high initial activity in addition to the ideal properties of MP EcoChrom™ Silica. The initial activity of MP EcoChrom™ Silica, active, corresponds to the activity of Alumina activity I.

With MP EcoChrom™ Silica, active, adsorption chromatography with silica becomes as easy as partition chromatography. Like MP EcoChrom™ Alumina, MP EcoChrom™ Silica, active, can be deactivated to well-defined lower activities using polar solvents. When using water as the deactivating agent, refer to the following table:

Deactivation of MP EcoChrom Silica, active						
Activity:	I	II	III	IV	V	
Silica, active	0	10	12	15	20	% water to be added

MP EcoChrom™ Silica are not only well suited for the separation of non-polar substances in non-polar solvents, but also for solvent purification and all other applications where it is necessary to remove small quantities of polar impurities from a non-polar matrix.

Product Name	pH Value approx.	Loss on Ignition approx, % (1000°C/12h)	Water Soluble Matter approx. %	Bulk Density approx. g/mL	Specific Surface approx. m²/g	Size	Cat. No.
Silica 18-32 µm, active 60 Å	7	< 5	0.2	0.4	500-600	10 g	0202749
						100 g	0202805
Silica 32-63 µm, active 60 Å	7	< 5	0.2	0.4	500-600	500 g	0202750
Silica 32-100 µm, active 60 Å	7	< 5	0.2	0.5	500-600	500 g	0202766
Silica 63-100 µm, active 60 Å	7	< 5	0.2	0.5	500-600	500 g	0202767
Silica 63-200 µm, active 60 Å	7	< 5	0.2	0.5	500-600	500 g	0202769
Silica 100-200 µm, active 60 Å	7	< 5	0.2	0.5	500-600	500 g	0202747
Silica 200-500 µm, active 60 Å	7	< 5	0.2	0.5	500-600	500 g	0202770

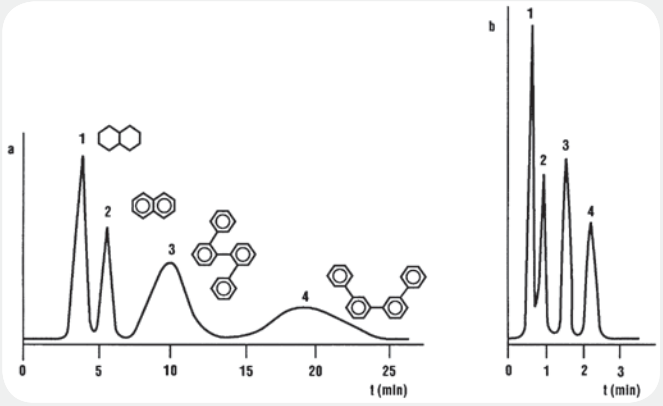
MP EcoChrom™ SiliTech for Industrial Applications

Product Name	Size	Cat. No.
SiliTech 0-32 µm, 60 Å	25 kg	0202780
SiliTech 12-26 µm, 60 Å	25 kg	0202774
SiliTech 18-32 µm, 60 Å	25 kg	0202778
SiliTech 32-63 µm, 60 Å	25 kg	0202071
SiliTech 63-200 µm, 60 Å	25 kg	0202066
SiliTech 200-500 µm, 60 Å	50 kg	0202068

Chromatography at Higher Pressure

Reducing the particle size increases the number of theoretical plates in a given column. More theoretical plates lead to better separations but also decrease eluent velocities. So, the gain in resolution is countered by longer separation times and, therefore, a zone broadening of the separated substances. Thus the gain in resolution may be negated by the long separation time.

Increasing column pressure reduces separation times, providing better separations in much shorter times, compared to classical open column chromatography. Improvement in the resolution and speed of a given separation are shown in the graph.



Clean and narrow sieve cuts are essential for chromatography with elevated pressure. Only closely cut particle size ranges can provide optimized column packing to achieve perfect separations. MP Adsorbents for MPLC and HPLC are optimally cut and have narrower particle size distributions than other supposedly smaller sieve cuts.

MP EcoChrom™ Alumina for MPLC

MP EcoChrom™ Alumina for Medium Pressure Liquid Chromatography (MPLC) are produced from the identical raw material as is used for MP EcoChrom™ Alumina Super I. Because of their unique adsorption properties and with their particle size, these products represent the link between the classical open column chromatography and the high performance chromatography. These Alumina may also be used for purification of small amounts of valuable material in semi-preparative scale procedures.

Product Name	pH Value approx.	Loss on Ignition approx, % (1000°C/12h)	Loss on Drying approx. % (180°C/2h)	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Alumina N 18-32 µm neutral pH	7.4	–	3.5	0.8	200*	10 g	0202056
						100 g	0202057
Alumina N 32-63 µm neutral pH	7.4	–	–	0.8	200*	500 g	0202061
						50 kg	0202026
Alumina A 18-32, active acid pH	4.5	< 1.7	–	0.8	200	100 g	0202063
Alumina B 18-32, active basic pH	10	< 1.7	–	0.8	200	100 g	0202065
Alumina N 18-32, active neutral pH	7.4	< 1.7	–	0.8	200	10 g	0202058
						100 g	0202059
Alumina B 32-63, active basic pH	10	< 1.7	–	0.8	200	500 g	0202040
						50 kg	0202041
Alumina N 32-63, active neutral pH	7.4	< 1.7	–	0.8	200	500 g	0202062
						50 kg	0202020

*when properly activated

MP EcoChrom™ Silica for MPLC

MP EcoChrom™ Silica for Medium Pressure Liquid Chromatography (MPLC) are irregular particles with a mean pore size diameter of 60 Å. They are produced from the identical raw material as is used for MP EcoChrom™ Silica designed for open column chromatography. Therefore, they show the same sorption behavior and allow easy switching between the methods.

MP EcoChrom™ Silica for MPLC are used for analytical and preparative separations. Their preparation performances on both the analytical and preparative scale are comparable to those of silica for HPLC. These silica are also used for semi-preparative industrial separations.

MP Bio also offers MP EcoChrom™ Silica, active, for MPLC for the adsorption chromatography.

Product Name	pH Value approx.	Loss on Ignition approx, % (1000°C/12h)	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Silica 12-26 µm, 60 Å	*7*	–	0.4	500-600*	100 g	0202735
					500 g	0202736
					1 kg	0202737
					2.5 kg	0202739
					25 kg	0202738
Silica 18-32 µm, 60 Å	*7*	–	0.4	500-600*	10 g	0202745
					100 g	0202757
					500 g	0202753
					1 kg	0202754
					2.5 kg	0202755
					25 kg	0202830
Silica 18-32 µm, active 60 Å	*7*	< 5	0.4	500-600	10 g	0202749
					100 g	0202805
Silica 32-63 µm, active 60 Å	*7*	< 5	0.4	500-600	500 g	0202750

*when properly activated

MP EcoChrom™ Alumina for HPLC

MP Bio is one of the few manufacturers of alumina for High Performance Liquid Chromatography (HPLC). MP Alumina for HPLC are produced from the identical raw material as is used for MP EcoChrom™ Alumina Super I and exhibit identical separation behavior.

- Successfully used to separate unsubstituted and substituted condensed aromatic compounds
- Recommended for the separation of ionic compounds, due to partition and/or adsorption chromatographic capacity and weak ion-exchange activity

Product Name	pH Value approx.	Loss on Drying approx. % (180°C/2h)	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Alumina N 3-6 µm neutral pH	7.4	3.5	0.8	200*	10 g	0202142
					100 g	0202143
Alumina N 7-12 µm neutral pH	7.4	3.5	0.8	200*	10 g	0202148
					100 g	0202149
Alumina N 10-18 µm neutral pH	7.4	3.5	0.8	200*	10 g	0202151
					100 g	0202152

*when properly activated

MP EcoChrom™ Silica for HPLC

The chromatographic behavior of MP EcoChrom™ Silica for High Performance Chromatography (HPLC) is identical to the behavior of MP EcoChrom™ Silica for Column Chromatography. The very closely controlled particle range guarantees dense column packings, high plate numbers and relatively low back pressure.

Besides its analytical use, MP EcoChrom™ Silica for HPLC is also frequently employed for technical chromatography.

Product Name	pH Value approx.	Loss on Drying approx. % (180°C/2h)	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Silica 3-6, 60 Å	7	3.5	0.3	500-600*	10 g	0202790
					100 g	0202791
Silica 7-12, 60 Å	7	3.5	0.4	500-600*	10 g	0202793
					100 g	0202794
Silica 10-18, 60 Å	7	3.5	0.4	500-600*	10 g	0202796
					100 g	0202797

*when properly activated

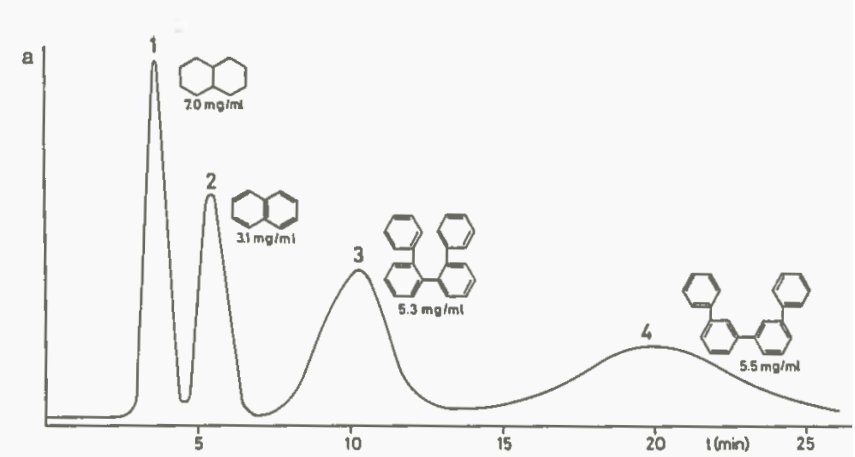
Alumina N 18

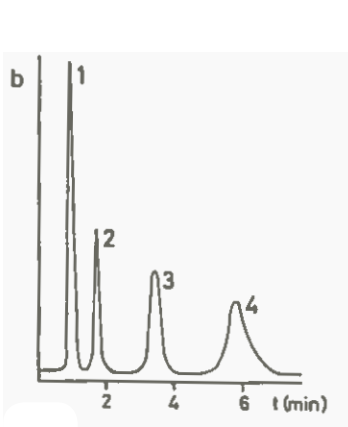
An Exact and Rapid Method for the Determination of Polycyclic Hydrocarbons

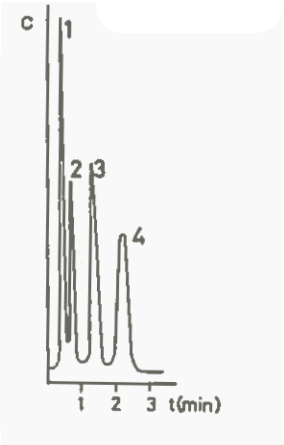
J.N. Little
Short Communication – Waters Associates Inc., 61 Fountain Street, Framingham, Mass. 01701/USA

The use of high speed analytic liquid chromatographs requires fine-grained adsorbents with narrow mesh cuts. With these adsorbents, the method can compete with thin-layer chromatographic separation regarding the time needed. This is demonstrated in diagrams *a*, *b* and *c*. Diagram *a* refers to Aluminum Oxide Woelm (now MP Aluminum Oxide) neutral, Act. I. Diagrams *b* and *c* refer to Alumina Woelm (now MP Alumina) N 18. The experimental details are shown in the table below. One can easily recognize that in cases *b* and *c* – though using shorter columns and only a fifth or a tenth of the time – an excellent separation of the polycyclic hydrocarbons was achieved.

	a	*b*	*c*
Column	Al_2O_3 Woelm neutral, Act. I (70-150 µ); 6% H_2O added (w/w)	Alumina Woelm N 18 (18-30 µ); 6% H_2O added (w/w)	Alumina Woelm N 18 (18-30 µ); 6% H_2O added (w/w)
Instrument	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 32 Attenuation	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 8 Attenuation	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 8 Attenuation
Dimensions of the column	100 cm x 2.3 mm inner diam.	50 cm x 2.3 mm inner diam.	20 cm x 2.3 mm inner diam.
Solvent	Hexane	Hexane	Hexane
Flow rate	0.82 mL/min	1.5 mL/min	1.5 mL/min
Injection volume	20 µL	20 µL	10 µL

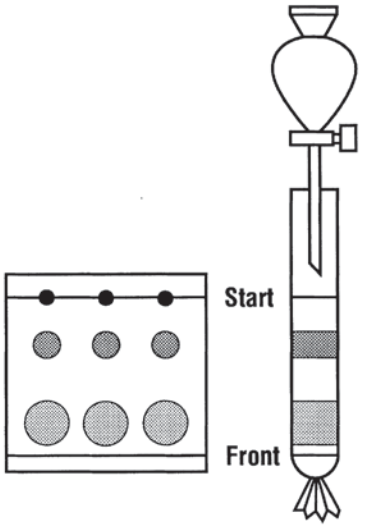






Dry Column Chromatography (DCC)

The Dry Column Chromatography (DCC) bridges the gap between analytical Thin-Layer Chromatography (TLC) and the production-oriented column chromatography (CC). In DCC, as in TLC, the solvent comes in contact with the adsorbent for the first time at the start of the development of the column. The sample is charged onto the dry column and the development is stopped when the solvent front reaches the end of the column. As in CC, columns of almost any size may be filled in DCC to separate or clean up very large quantities of materials. The length of the column does not play the same roll as the migration length in TLC because of a completely different solvent transport mechanism: In TLC, migration by capillary attraction competes against gravity; in DCC, the flow is forced by the gravity itself.



DCC is often used to scale up TLC separations. However, to successfully convert a thin-layer chromatographic separation to the dry column, it is imperative to imitate the TLC parameters as closely as possible. The adsorbent used on TLC plates, exposed to air and humidity, usually has an activity of II-III according to the Brockmann and Schodder scale.

MP EcoChrom™ adsorbents for Dry Column Chromatography are therefore adjusted to this activity. An important parameter for each separation is the particle size of the adsorbent used. The particle sizes of MP EcoChrom™ adsorbents for DCC are adjusted to give optimized separations.

TLC plates are often prepared with a UV phosphor to make uncolored substances visible. MP EcoChrom™ adsorbents for DCC also include a fluorescent indicator with an excitation at 254 nm.

MP EcoChrom™ adsorbents for DCC imitate the chemical and physical properties of Thin-Layer Chromatography as closely as possible. All adjustments and fine tunings are made during the production of the DCC adsorbents. MP EcoChrom™ adsorbents for DCC can be used without any further preparation. Columns may be filled directly from the bottle in which the adsorbent was supplied. Occasionally, the DCC separation will not correspond closely enough to those previously achieved in TLC. This may occur if the TLC separation was made with a pre-saturation of the TLC plate. In this instance, the DCC separation may be improved by mixing the adsorbent with about 10% of its weight in a solvent mixture corresponding to the vapor phase formed over the solvent (-mixture) in the TLC tank. Using MP EcoChrom™ adsorbents for TLC and MP EcoChrom™ adsorbents for DCC improves the scale-up from TLC to DCC because both are made from the same raw material.

MP EcoChrom™ DCC Adsorbents

Product Name	pH Value approx.	Activity acc. to Brockmann	Bulk Density approx. g/mL	Size	Cat. No.
Alumina DCC particle size: 63-200 µm contains Flourescent Indicator F254 nm	7.4	III	0.8	500 g	0204512
				5 kg	0204511
				50 kg	0204514
Silica DCC, 60 Å particle size: 63-200 µm	7	III	0.5	500 g	0204524
				3 kg	0204526
				25 kg	0204530
Nylon Foil Tubing, 40-44 mm flat diameter				20 meter	0209653
DCC Package 1: Alumina Contents: 1 x 500 g Alumina DCC, 1 x 20 m Nylon Foil Tubing, Step-by-Step working guide				1 package	0204516
DCC Package 2: Silica Contents: 1 x 500 g Silica DCC, 1 x 20 m Nylon Foil Tubing, Step-by-Step working guide				1 package	0204532

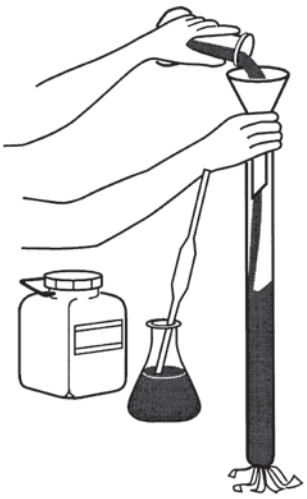
DCC is a "non-elution" method of column chromatography, which means that the separated substances remain in the column at the end of the chromatographic process. The isolation of the separated substances can be carried out using Nylon Foil Tubings instead of glass or steel columns. Nylon Foil Tubing can easily be cut into appropriate segments. The following instructions for DCC assume the use of a Nylon Foil Tubing.

1 Preparation of the column

The foil tubing comes as a flat ribbon of 40-44 mm width. This forms a column of approx. 25 mm diameter. 10 cm of this tubing can hold around 6.5 grams of MP EcoChrom™ Silica DCC or 13 grams of MP EcoChrom™ Alumina DCC.

Add 10 cm to the desired length and cut this amount of foil. Close one end of the foil by sealing it in the flame of a gas lighter, folding and stapling, tying or any other suitable method. To remove the folds of the foil, fill the closed-end tubing with very hot water, rinse with acetone, and dry with hot air. Insert a pad of cotton or glass wool into the closed end of the tubing and pierce several times with a large needle to allow the air to escape when the solvent migrates through the column.

2 Filling the column



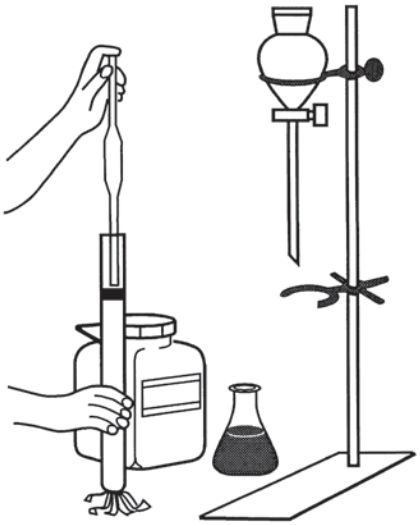
Pour the adsorbent into the prepared column directly from its container, or after conditioning with the solvents (-mixture). Add a small amount at a time, gently tapping the column or apply a vibrator to compact the column.

A well-filled column should stand up by itself and may be held with a simple laboratory clamp.

3 Preparation of the sample

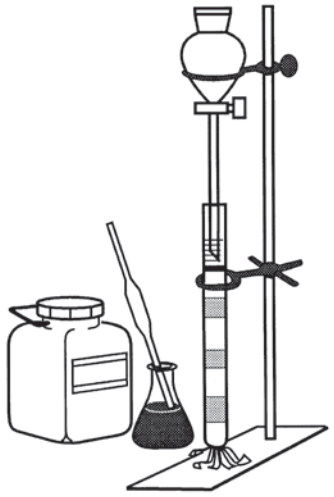
Add a few mL of the sample solution to about 1 g of the appropriate adsorbent. The solvent is then evaporated using a rotation evaporator until sample and adsorbent form a free flowing powder. Occasionally, a highly concentrated solution of the sample may be used. In this case, the solvent used in the sample solution should be identical with the solvent (-mixture) used for the separation on TLC.

4 Charging the column



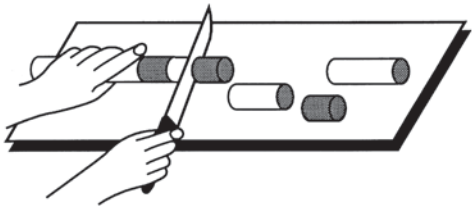
Apply the adsorbate of the sample as a level layer on the top of the column. Cover this layer with about 2 cm of pure adsorbent. If a concentrated sample solution is to be applied, care should be taken to achieve a narrow starting zone. However, applying liquid sample causes more uneven zones than applying the sample as an adsorbate. After penetration of the solution into the top of the column filling, about 2 cm of pure adsorbent should be added.

5 Development of the column



Carefully add the solvent (-mixture) that formed the best results in the preliminary TLC experiments to the top of the column with a separation funnel. The solvent should migrate slowly and steadily through the column. It has been shown that a constant liquid head of about 1-2 cm provides optimal results in DCC separation. A constant liquid head is attained by placing the end separation funnel about 1 cm over the top of the column. Depending on length of the column, the viscosity of the solvent (-mixture), and the height of the liquid head, the development of the column should be finished within about 15 to 30 minutes. As with TLC, the components of the initial sample mixture should be separated from each other on the column. The solvent volume applied to the top of the column should not exceed the amount which is necessary to fill the void fraction of the adsorbent bed.

6 Recovery of the separated compounds



After development, immediately place the column flat on an appropriate surface. Mark the separated zones on the column, using UV light, if necessary. Cut the column into sections with a very sharp knife (cut without applying excessive pressure to the column). The adsorbent of a well packed column should be able to be cut into small slices without crumbling.

Place the cut slices into Buchner funnels and extract and process by appropriate methods.

If the desired compounds are not colored and inactive in UV light, or, if aromatic solvents have been used, a detection of the separated compounds may be achieved according to the R1-value, obtained in a preliminary TLC-separation. The R1-values of the DCC usually correspond to those of the TLC.

They are calculated as usual:

$$R_1 = \frac{L_3}{L_1}$$

L_3 = Length: top of the column to center of the compound

L_1 = Length: top of the column to solvent front

If the column is cut "blind" according to the R1-values, it is recommended to cut a broad section around the expected center of the substance zone and cut one or two small slices on each side of this broad section. The different cuts of the column are processed separately and a TLC of each of the fractions shows the content of the desired compound. Blind cuts of the column should only be used if the difference of the R1-values of two neighboring substances is 0.1 or greater.

If TLC is performed with MP ready-to-use TLC-plates or with TLC-plates prepared with MP EcoChrom™ adsorbents for TLC, then the adsorbents for TLC and the adsorbents for DCC are made from the same raw material. This gives better results in scaling-up from TLC to DCC than the use of adsorbents of different origins.

Pre-Separation of Essential Oils and Similar Complex Substance Mixtures for GC-Analysis by Means of Dry-Column Chromatography

Kubeczka, K.-H. *Chromatographia.* **1973**, *6* (2).

Despite the high efficiency of gas chromatographic procedures, it is frequently not possible to achieve complete separation of all components in the case of complex mixtures of natural substances, such as essential oils. A pre-adsorptive separation by means of column chromatography offers a solution to the problem, in particular because this method excludes certain substance groups from the start, due to the different polarities of the individual fractions.

This useful procedure ensures to a high degree the avoidance of frequently occurring artifacts and allows a preliminary separation into 5 fractions of different polarity. Figure 1 shows that the slightly polar fractions 1 and 2 are obtained by elution with pentane or benzene, using a silica gel column for dry-column chromatography; the polar fractions of the mixture are obtained by cutting-up of the column-filling into 3 parts and subsequent elution with ether/methanol 8 + 2 (v/v). This results in a separation into 5 fractions of different polarity. With the application of the standardized silica gel for dry-column chromatography with adjusted water content, additional deactivation of the sorbent is not necessary. Several experiments conducted showed no evidence of significant loss or changes of the compounds under test.

The equipment consists of a chromatographic tube of 250 mm length. The lower end of the tube is closed by means of a ground glass-adapter with a fused sintered glass filter and a one-way stopcock with fused vacuum outlet piece is provided. Ground round bottom flasks are used as receivers.

The adsorption tube is packed with Silica Gel Woelm (now MP Silica) for Dry-Column Chromatography, which is compacted by vibration to a height of 10 cm. 2 mL of a 10% solution of the essential oil in n-pentane or n-hexane is applied on top of the column and covered by a 1 cm-layer of the silica gel mentioned above. This is followed by elution with 150 mL of n-pentane, which must completely pass through the column (= fraction 1). Then, the receiver is changed and development is carried out by means of 65 mL of benzene (= fraction 2). When the column runs dry, it is divided – starting from the bottom – in a 4:3:3 ratio; the individual silica gel-zones are scratched out with a spatula and each one is immediately suspended in a 10 mL portion of a mixture of ether methanol (8+2). By this, fractions 3, 4 and 5 are obtained.

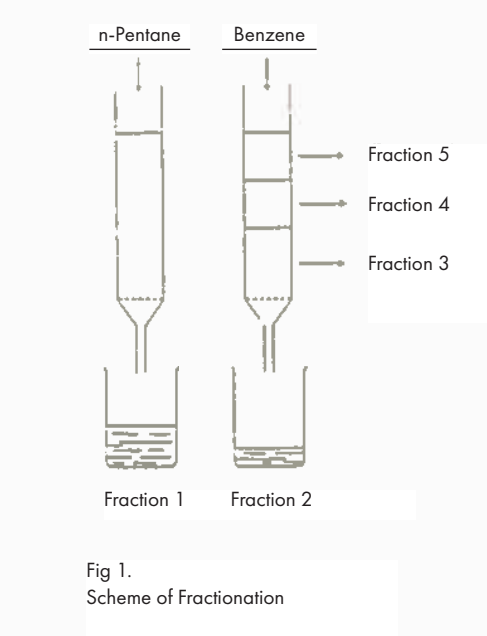
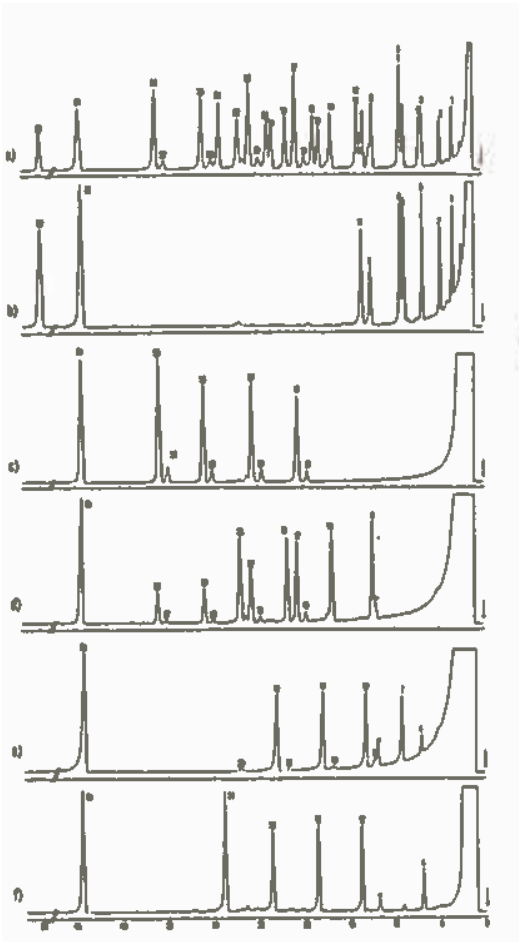


Fig 1.
Scheme of Fractionation

The fractions 3-5 are processed separately according to the following schedule: After transferring the suspension into the chromatographic apparatus described above, allow the liquid to drain off, wash with 30 mL of an ether-methanol-mixture (8+2), extract twice with 40 mL of water to remove the methanol, and dry the ether-phase by means of Na_2SO_4. Concentrate the fractions to 2 mL under reduced pressure and N_2-atmosphere.

Fraction 1 contains the apolar compounds (hydrocarbons). Fraction 2 comprises the slightly polar compounds, such as esters of monocarboxylic acids of medium length chain. Compounds found in fraction 3 are, for instance, acetates of aliphatic alcohols of medium length chain. Fraction 4 contains aliphatic aldehydes and ketones, and fraction 5 the most polar compounds (alcohols, etc).

Figure 2 provides an example for the separation of a test-mixture of the components of essential rue oil by means of gas chromatography. Apart from a simplification regarding the composition of samples, this procedure in many cases facilitate the identification of individual components.



a = total test mixture
b = fraction 1
c = fraction 2
d = fraction 3
e = fraction 4
f = fraction 5

Marking of Peaks
1 = α-Pinene
2 = β-Pinene
3 = Limonene
4 = Cineol
5 = Terpinolene
6 = p-Cymene
7 = 2-Octanone
8 = 2-Octyl acetate
9 = cis-allo-Ocimane
10 = 2-Nonanone
11 = trans-allo-Ocimene
12 = 2-Octanol
13 = 2-Nonyl acetate
14 = 2-Decanone
15 = 2-Nonanol
16 = 2-Octyl-2-methyl butyrate
17 = 2-Octyl-3-methyl butyrate
18 = 2-Decyl acetate
19 = 2-Undecanone
20 = 2-Decanol
21 = 2-Nonyl-2-methyl butyrate
22 = 2-Nonyl-3-methyl butyrate
23 = 2-Undecyl acetate
24 = 2-Undecanol
25 = 2-Decyl-2-methyl butyrate
26 = 2-Decyl-3-methyl butyrate
27 = 2-Undecyl-2-methyl butyrate
28 = 2-Undecyl-3-methyl butyrate
29 = Guaiazulene

St = Standard (2-Pentadecanone)
* = Diacetone alcohol

Fig 2.
Gas chromatogram of different fractions of a test mixture

Al_2O_3 for Dry-Column Chromatography
SiO_2 for Dry-Column Chromatography

Isolation of Pure Substance from Reaction Products by Dry-Column Chromatography

W. Kühnle

Brief communication – Max-Planck-Institut für Biophysikalische Chemie.
Abl. Spektroskopie, D34 Göttingen-Nikolausberg, Am Faßberg.

Reaction products can be separated or organic substances purified by dry-column chromatography (DCC). Substance mixtures which do not show any migration or cannot be separated on the TLC-plate are also inseparable when using dry-column chromatography. At present, the separation of substance mixtures is restricted to those which can be made perceptible under UV-light, because the separated substances in the dry-column may not be rendered visible by means of a spraying reagent. The following table shows the isolation of some substances from reaction mixtures on Alumina Oxide and Silica Gel Woelm (now MP Alumina and Silica) for Dry-Column Chromatography. The substances obtained yield good elementary analysis values. A recrystallization of the separated products was only necessary in rare instances.

The following solvent systems were used:

1. Cyclohexane/ethyl acetate 1:1
2. Diethyl ether/methanol 9:1
3. Cyclohexane/carbon tetrachloride 1:1
4. Cyclohexane/ethyl acetate 95:5
5. Benzene/ethyl acetate 95:5
6. Cyclohexane/ether 1:1
7. Cyclohexane/benzene 1:1
8. Chloroform/acetone 8:2
9. Cyclohexane/ethyl acetate 7:3
10. Benzene/methanol 8:2
11. Chloroform/benzene 1:1
12. Benzene/methanol 9:1
13. Hexane/ether 7:3

Isolated Substances	DCC Adsorbent	Solvent
p-Ethoxybenzyl-p-n-butylphenylketone	Al_2O_3	1
	SiO_2	2
1-(p-Ethoxyphenyl)-2-chloro-2-p-n-butylphenylethylene	Al_2O_3	3
1-(9-Anthranoyl)-2-(p-N,N-dimethylaminophenyl)ethane	Al_2O_3	4
1-(9-Anthryl)-3-(p-N,N-dimethylaminophenyl)propane	Al_2O_3	4
3-(9-Anthracene)propanol-1	Al_2O_3	5
9-Anthraceneethylchloride	Al_2O_3	5
N-(9-Anthrylethyl)-N-methylaniline	Al_2O_3	5
N-(9-Anthryl-n-propyl)-N-methylaniline	Al_2O_3	5
4-Biphenylaldehyde	SiO_2	6
1-(4-Biphenyl)-3-(pentamethylphenyl)propane	Al_2O_3	7
(3-Cholesteryl)-[4-(2,2,6,6-tetramethylpiperidinyloxide)]carbonate	SiO_2	6
1-(p-Carboxyphenyl)-3-(p-N,N-dimethylaminophenyl)propane ethylester	Al_2O_3	6
1-(p-Cyanobenzoyl)-2-(p-N,N-dimethylaminophenyl)ethane	Al_2O_3	9
1-(N,N-Dimethylamino)-2-(p-cyanophenyl)ethane	SiO_2	9
α-N,N-Diethylamino-p-tolunitrile	SiO_2	10
1-(N,N-Diethylamino)-3-(p-cyanophenyl)propane	Al_2O_3	10
4-N,N-Dimethylamino-2-methylbenzonitrile	Al_2O_3	9
4-N,N-Dimethylamino-2-methoxybenzonitrile	Al_2O_3	11
4-N,N-Dimethylamino-3-methylbenzonitrile	Al_2O_3	9
p-N,N-Dimethylaminophenylethylbromide-2	Al_2O_3	6
p-N,N-Dimethylaminophenyl-n-propylbromide-3	Al_2O_3	6
p-N,N-Dimethylaminobenzoic acid-9-anthryl-methylcarbinolester	Al_2O_3	5
4-Methoxy-N-methyldiphenylamine-4'carboxylic acid methylester	Al_2O_3	12
4-Methoxy-4'-n-butylazobenzene	SiO_2	6
3-(1-Naphthyl)-n-propyl-N,N-diethylamine	Al_2O_3	5
2-(1-Naphthyl)ethyl-N,N-diethylamine	Al_2O_3	5
4-(1-Naphthyl)-n-butyl-N,N-diethylamine	Al_2O_3	5
12-[3(N-Oxyl-4,4'-dimethyloxazolidine)]stearic acid methyl ester	SiO_2	13
4,4',4''-Trimethoxytriphenylamine	Al_2O_3	4
1,3-Di[1,1-pyrenyl]propane	Al_2O_3	7
1-[Pyrene]-3-[4-N,N-dimethylaminophenyl] propane	Al_2O_3	9
1,3-Di[1,1-naphthyl] propane	Al_2O_3	3
N,N-Dimethylaniline-d11	Al_2O_3	6
4-n-Octoxy-4'-methoxy-α-stilbene	Al_2O_3	6
2-[1-Pyrene]ethanol	SiO_2	1
2-[1-Pyrene]ethyl-1-bromide	Al_2O_3	6

Thin-Layer Chromatography (TLC)

Thin-Layer Chromatography (TLC) is a quick chromatographic method for qualitative screening and for quantitative determinations.

Advantages:

- Inexpensive equipment for qualitative and semi-qualitative separations
- Simple handling
- Short separation time
- Low solvent consumption
- Low detection limits
- Simultaneous separation of a large number of samples on the same plate

Usually, qualitative and semi-quantitative separations are evaluated visually. If necessary, a suitable staining procedure may be used by spraying the dried TLC-place with appropriate reagents. Quantitative separation requires some equipment, but is still quick and in many cases, more economic than other methods, e.g. HPLC. The accuracy of modern evaluation equipment is comparable to that of HPLC.

The advantages of modern TLC have led to a revival of this method in recent years. MP Bio offers a large range of adsorbents to prepare TLC-plates as well as ready-to-use precoated plates.

MP EcoChrom™ Alumina for TLC

MP EcoChrom™ Alumina for Thin-Layer Chromatography are produced from the same raw material as MP EcoChrom™ Alumina Super I for Column Chromatography. They are available with the same three surface modifications: basic, neutral, and acid. With MP EcoChrom™ Alumina for TLC, it is easy to optimize the separation method to achieve the best fit for the separation problem. MP EcoChrom™ Alumina can be used for adsorption and/or partition chromatography, and also ion-exchange chromatography.

The addition of inorganic fluors, e.g. Tl -activated zinc silicate, allows the preparation of fluorescent layers to enable the detection of uncolored compounds by UV quenching.

Except for MP EcoChrom™ Alumina G-TLC, MP EcoChrom™ Alumina for TLC does not contain any binder. The user is free to choose the binder which is best suited for the desired application.

MP EcoChrom™ Alumina G-TLC contains 11% gypsum. To prepare five 20 x 20 cm plates with 250 µm layers, about 35 g MP EcoChrom™ Alumina G-TLC are mixed with approximately 40 mL water to form a slurry. This slurry may be spread on the plates using a commercially available spreading device.

The compounds that have been separated on alumina layers are provided on the next page.

Classes of compounds separated on alumina thin-layers	
Alcohols	Polyalcohols of short or medium chain length
Alkaloids	Purine-, pyridine-, isoquinoline-, indole-alkaloids
Amines	Primary, secondary, and tertiary long-chain amines, isomers of nitroaniline
Amino acids, Peptides, Proteins	Sodium salts of amino acids, dicarbobenzoxy (Cbz) amino acids, t-butyloxycarbonyl (BOC) amino acids
Carbohydrates	Sugars and their phenylhydrazone compounds
Carbonyl compounds	Diketones, hydroxyaldehydes, 2-hydroxy benzophenone, chloramphenicol alkylated cyclohexanols, carbonyl-2,4-dinitrophenyl hydrazone
Dyes and Pigments	Liposoluble food dyes, indicator dyes, azo dyes, Cr- and Co-chelates of azo and azomethine dyes, cosmetic pigments, anthraquinone and amino-anthraquinone pigments, carotenes, coumarins, pteridines
Compounds with heterocyclic N	Decahydroquinoline, perhydropyridine, pyrazole, polynuclear carbazoles pyridine
Compounds with P and/or S	Esters and amides of thiophosphoric and pyrophosphoric acid, thiophene derivatives, thiopyrons
Hydrocarbons	Unsubstituted and substituted polycyclic aromatic compounds
Pesticides	Chlorinated hydrocarbons, organic thiophosphates
Pharmaceuticals	Antihistaminics, barbituates, local anaesthetics, sulfonamides, cardiac glycosides, glutarinides
Sulfonates	Alkyl-aryl-sulfonates, xylene and toluene sulfonates
Stabilizers	2-hydroxy-benzophenone

Product Name	pH Value approx.	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Alumina A-TLC acid pH, 5-25 µm	4.5	0.8	200*	1 kg	0204346
				50 kg	0204347
Alumina B-TLC basic pH, 5-25 µm	9	0.8	200*	1 kg	0204340
				50 kg	0204341
Alumina N-TLC neutral pH, 5-25 µm	7.4	0.8	200*	1 kg	0204343
				50 kg	0204344
Alumina G-TLC neutral pH, 5-25 µm contains gypsum	7.4	0.8	200*	1 kg	0204409
				50 kg	0204413

*when properly activated

MP EcoChrom™ Silica for TLC

MP EcoChrom™ Silica for Thin-Layer Chromatography are produced from the same highly standardized raw material as the MP EcoChrom™ Silica for Column Chromatography. These silica are available with or without binder and with or without fluorescent indicators. Thus, the user is free to choose whichever type best fits the specific separation.

MP EcoChrom™ Silica G-TLC and MP EcoChrom™ Silica GF-TLC contain 11% gypsum. To cover five 20 x 20 cm plates with a layer of about 300 µm thickness, about 25 g MP EcoChrom™ Silica G-TLC or MP EcoChrom™ Silica GF-TLC are stirred with approximately 40 mL water. The slurry is spread on the plates using a commercially available spreading device.

Product Name	pH Value approx.	Bulk Density approx. g/mL	Specific Surface approx. m^2/g	Size	Cat. No.
Silica TLC, 60 Å particle size: 5-15 µm	7	0.4	500-600*	500 g	0204642
				25 kg	0204671
Silica F-TLC, 60 Å particle size: 5-15 µm contains Fluorescent Indicator F254 nm	6.5	0.4	500-600*	500 g	0204643
Silica G-TLC, 60 Å particle size: 5-15 µm contains gypsum	6.5	0.4	500-600*	500 g	0204644
				25 kg	0204674
Silica GF-TLC, 60 Å particle size: 5-15 µm contains Fluorescent Indicator F254 nm and gypsum	6.5	0.4	500-600*	500 g	0204645
				25 kg	0204680

*when properly activated

MP EcoChrom™ Polyamides

MP EcoChrom™ Polyamide for Chromatography is based on Nylon 6 (poly-[ε-aminocapro-lactam]). The amino- as well as the carbonyl function of the peptide bond are able to achieve very stable hydrogen bonds. The strongest hydrogen bond is due to the phenolic proton, reinforced by further hydroxyl groups in the meta- and/or para-position and weakened by hydroxyl groups in the ortho-position. Aromatic carbon acids are bound in the same manner, as the aromatic moiety is larger and forms stronger bonds. Nitro compounds may be bound either by hydrogen bond or by Lewis acid-base reaction. Thus, for the elution of nitro compounds, weak buffer solutions are useful.

MP EcoChrom™ Polyamides for Chromatography are available for Column Chromatography as well as for Thin-Layer Chromatography. Both types are produced from the same raw material. This allows easy transfer between Thin-Layer and Column Chromatography.

Due to its outstanding properties, MP EcoChrom™ Polyamide for Chromatography is well suited for the purification of plant tissue extracts. Phenols may be easily removed from such extracts.

MP EcoChrom™ Polyamide for Chromatography exhibits constant selectivities towards:

Phenols, aromatic nitro and amino compounds	Chalcones, chinones, flavones	Anthraquinones
DNP-amino acids	Carbonic acids and their amides	Sulfonic acids and their amides

Product Name	pH Value approx.	Bulk Density approx. g/mL	Size	Cat. No.
Polyamide particle size: 50-160 µm	7	0.2	250 g	0209602
Polyamide TLC particle size: 5-20 µm	7	–	250 g	0209603

Dehydration of Organic Solvents

Modern preparative and analytical chemistry requires more anhydrous solvents with the lowest residual water content possible. Commercially available so called "anhydrous" or "dried" solvents usually contain up to 0.01% (10 ppm) water. They may be called "water reduced." These solvents have to be dehydrated if they are to be used in HPLC, spectroscopy, and voltametry. Dehydrating agents may be divided into two basic groups: Chemical dehydrating agents and Physical dehydrating agents.

Chemical Dehydrating Agents

Compounds which either absorb the water as water of crystallization (e.g. calcium chloride, sodium sulfate), or remove it by reaction (e.g. phosphorous pentoxide, alkaline metals or alkaline- and alkaline earth metal hydrides). For a compound to qualify as a desiccant it has to meet the following requirement: Neither the compound itself nor its reaction products with the water may be solvent soluble, as they would have to be removed from the dehydrated solvent. However, even so called "insoluble" compounds are often marginally soluble.

Physical Dehydrating Agents

Materials which either adsorb the water on their surface (e.g. alumina, desiccant silica) or enclose the water in pores (molecular sieves). The advantage of these physical dehydration agents are that they are almost completely insoluble and, due to this fact, can be easily separated from the dehydrated solvent. Also, physical dehydration agents are easy to handle and regenerate. The easiest method is to pass the solvent to be dehydrated through a column filled with the dehydrating agent. The water will be retained and the pure, dehydrated solvent will leave the column. Compared with chemical dehydration agents, the dehydration power of physical dehydration agents is rather poor. With desiccant silica, as well as with molecular sieves, a residual water content of between 0.001% (10 ppm) and 0.01% (100 ppm) can be reached.

Dehydration of Solvents with MP EcoChrom™ Alumina B - Super I

With the introduction of the super-active alumina by Woelm (Alumina WOELM W200 = MP EcoChrom™ Alumina Super I) a dehydration agent became available to the market which combines the advantages of physical dehydrating agents (easy to handle and regenerate) and chemical dehydrating agents (highest dehydration power). Outstanding results may be achieved with simple filtration through a column.

The dehydrating capacity (mL of the water-saturated solvent at room temperature, which can be dehydrated by 1 g of MP EcoChrom™ Alumina B - Super I to a residual water content of less than 2 ppm) determined by Santelmann for some commonly used solvents are listed in the following table:

Solvents which were dehydrated to a water content less than 2 ppm			
Solvent	Water content ppm	Solvent / Alumina Break through by	
		Weight (g/g)	Volume (mL/g)
n-Hexane	100	1450	2190
Cyclohexane	130	1630	2090
Decalin	700	1400	1580
Frigen 113 CR	130	1770	1130
Isooctane	130	660	950
Tetrachloroethylene	200	1440	880
Tetrachloromethane	100	1150	720
Ethylbenzene	400	290	330
Isopropylbenzene	300	280	320
Trichloroethylene	250	380	260
Bromobenzene	-	380	250
Chlorobenzene	400	250	220
Benzene	650	170	190
DL-Limonene	-	70	80
Trichloromethane	700	90	60
Dichloromethane	2000	55	41
Diisopropylether	7000	28	39
Acetonitrile*	1000	20	25
Dioxane*	1000	25	24
Tetrahydrofuran*	1000	21	24
Diethylether	14700	14	19

* water soluble solvent, adjusted to 0.1 % water

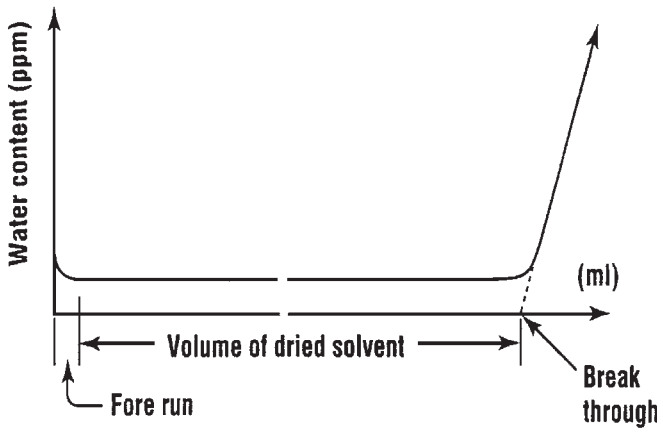
The experiments were performed on a column (15 mm inner diameter, approx. 30 mm height) filled with 5 g of MP EcoChrom™ Alumina B -Super I. The residual water content of the solvent leaving the column was continuously measured by a Panametrics hygrometer (Model 1000). The manufacturer states the detection limit of the hygrometer to be less than 1 ppm of water. In this range, however, the sensitivity is very low. Considering the signal/noise ratio, the detection limit was set to 2 ppm of water. A thin layer of granulated, dehydrated copper sulfate at the lower end of the column served as moisture indicator. Due to residual moisture in the connection parts between the column and the detector cell, a slight increase of the electric moisture signal may be noted when the first few milliliters of the dehydrated solvent enter the dried detector cell.

Dehydration of Solvents

These may be added to the top column again. This can also be done if the initial dehydrated solvent is turbid due to fines of alumina washed from the column. The presence of water is shown by a sudden rise of the electric moisture signal of the recorder (breakthrough). The copper sulfate as a moisture indicator shows a 10% higher breakthrough volume than the electric signal of the hygrometer due to the insensitivity of the optical reading and because the waterfront sags in the center of the column.

The following groups of solvents can be dehydrated with MP EcoChrom™ Alumina B-Super I:

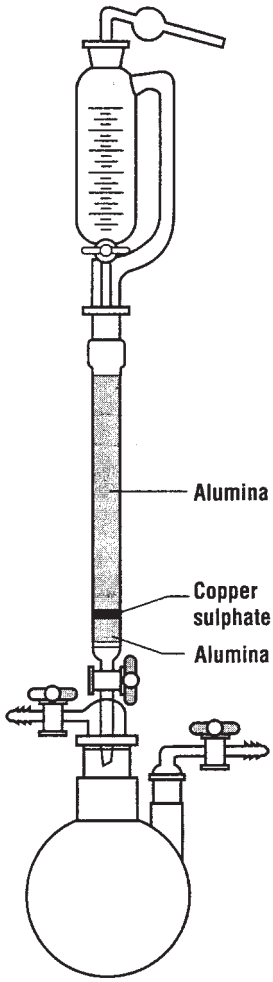
- Saturated and unsaturated hydrocarbons
- Saturated and unsaturated halogenated hydrocarbons (including cyanides)
- Unsubstituted, alkylated and halogenated aromatic compounds
- Cycloalkanes
- Ethers



The residual water content of dimethyl sulfoxide (DMSO) and pyridine could not be determined with the instrumentation described. Copper sulfate as an optical indicator could not be used due to the formation of colored complexes, partially soluble in the solvent. However, no decomposition can be shown by spectroscopic analysis.

Compounds having epoxy-, carbonyl-, orthio- groups cannot be dehydrated with MP EcoChrom™ Alumina B – Super I. Alcohols cannot be dehydrated by MP EcoChrom™ Alumina B – Super I due to their high polarity. To dehydrate solvents with MP EcoChrom™ Alumina B – Super I, the apparatus shown in the diagram should be used.

Estimating the water uptake capacity of the alumina at approximately 10%, the necessary amount of MP EcoChrom™ Alumina B – Super I can be calculated. The column should be dry filled with about 10% of MP EcoChrom™ Alumina B – Super I, followed by an approx. 2-3 mm thick layer of dehydrated copper sulfate, then covered with the remaining alumina. Ground coating the layer of copper sulfate moisture with about 10% of the alumina guarantees that the solvent is dehydrated and the alumina is used efficiently.



Before adding the solvent, the receiver vessel must be dried with a suitable protective gas, which may be passed through a drying tube, filled with alumina. The column can then be very slowly – drop by drop – fed with the solvent. During this initial step the column should not warm up perceptibly. After the column is completely wet, both stopcocks may be fully opened. To accelerate the throughput, a vacuum may be applied to the receiver vessel or hydrostatic pressure may be applied to the top of the column. Flow velocities up to 40 mL/minute do not affect the breakthrough volume or the residual water content.

If large quantities of dehydrated solvents are needed, continuous flow methods may be used. Shutoff times should be minimized and not exceed a few hours, as longer shut-off times may cause a decrease in quality and yield. Also the shelf life of the column may be reduced.

MP EcoChrom™ Adsorbents Selection Guide

Column Chromatography and Filtration

HPLC / MPLC

Particle Size	Alumina / Aluminum oxide	Page	Silica	Page
200-500 µm			Silica 200-500, 60 Å	15
			Silica 200-500, 60 Å active	16
			SiliTech 200-500, 60 Å	16
100-200 µm			Silica 100-200, 60 Å	15
			Silica 100-200, 60Å active	16
63-200 µm	Alumina B - Super I	4	Silica 63-200, 60 Å	15
	Alumina N - Super I	4	Silica 63-200, 60 Å active	16
	Alumina A - Super I	4	SiliTech 63-200, 60 Å	16
	Alumina B, Act. I	7	Silica DCC	21
	Alumina N, Act. I	7		
	Alumina A, Act. I	7		
	Alumina Act. II-III	7		
	Alumina DCC	21		
	Alu-oxide basic techn. A	9		
	Alu-oxide basic techn. A -H 32001-	9		
	Alu-oxide techn. -H 31101-	9		
	Alu-oxide neutral -H 15152-	9		
	Alu-oxide acid techn. A	9		
63-100 µm			Silica 63-100, 60 Å	15
			Silica 63-100, 60 Å active	16
32-100 µm			Silica 32-100, 60 Å	15
			Silica 32-100, 60 Å active	16
32-63 µm	Alumina B 32-63, active	17	Silica 32-63, 60 Å	14
	Alumina N 32-63	17	Silica 32-63, 60 Å active	16, 18
	Alumina N 32-63, active	17	SiliTech 32-63, 60 Å	16
18-32 µm	Alumina B 18-32, active	17	Silica 18-32, 60 Å	14, 18
	Alumina N 18-32	17	Silica 18-32, 60 Å active	16, 18
	Alumina N 18-32, active	17	SiliTech 18-32, 60 Å	16
	Alumina A 18-32, active	17		
12-26 µm			Silica 12-26, 60 Å	15, 18
			SiliTech 12-26, 60 Å	16
10-18 µm	Alumina N 10-18	19	Silica 10-18, 60 Å	19
7-12 µm	Alumina N 7-12	19	Silica 7-12, 60 Å	19
3-6 µm	Alumina N 3-6	19	Silica 3-6, 60 Å	19
0-63 µm			Silica 0-63, 60 Å	14
0-32 µm			SiliTech 0-32, 60 Å	16
1-3 mm	Alu-oxide 1-3 mm	9		

Product		
Dioxin Analysis	Alumina B-Super I f. Dioxin Analysis	Silica 63-200, 60 Å active
Isotope Technique	Alumina R	
Dry-Column Chromatography	Alumina DCC	Silica DCC — Accessories: Nylon-Foil-Tubing
Purification of Solvents	Alumina B - Super I (e.g. removal of peroxides, dehydration of solv., purification for UV-Spectroscopy) Alumina A - Super I (e.g. purification of acetonitrile) Alumina B, Act. I (e.g. removal of ethanol from chloroform, purification for UV-Spectroscopy) Alumina N, Act. I and Alumina A, Act. I (e.g. purification of solvents for UV-Spectroscopy) Silica 63-200, 60 Å active (e.g. purification of solvents for UV-Spectroscopy)	
Thin-Layer Chromatography	Alumina B-TLC Alumina G-TLC Alumina N-TLC Alumina A-TLC	Silica TLC Silica G-TLC Silica F-TLC Silica GF-TLC
Polyamides	Polyamide for Column-Chromatography + Polyamide TLC for Thin-Layer-Chromatography	

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