Bijlage 1

Rapid Determination of Color Additives, Using the C_{18} Cartridge

MARY L. YOUNG
Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

A reliable method has been developed for the rapid separation and identification of the 7 permitted FD&C dyes (Red Nos. 3 and 40; Blue Nos. 1 and 2; Yellow Nos. 5 and 6; Green No. 3) and the recently banned FD&C Red No. 2 in foods. The colors are separated by using the C_{18} cartridge, and their identity is confirmed by spectrophotometry.

The present methodology used for the separation and identification of synthetic colors in foods and drugs (1) is a long, tedious process involving 2 lengthy column chromatographic steps with Celite and cellulose. We describe a method based on reverse phase chromatography, using C_{18} cartridges for the separation of these colors.

The C_{18} cartridge (2) employs the principles of liquid chromatography to isolate, clean up, and concentrate sample components for final determination by visible spectrophotometry (3), thin layer chromatography (TLC) (4), or liquid chromatography (5). It accomplishes in a single step what usually requires a sequence of several time-consuming operations.

We report our results for the rapid determination of color additives in candy, beverage syrup, gum balls, pudding and pie filling mix, and cologne.

Experimental

Apparatus and Reagents

(a) *C_{18} cartridges.*—Sep-Pak (Waters Associates, Inc., Milford, MA).

Received May 9, 1983. Accepted March 15, 1984.
This report of the Associate Referee was presented at 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, D.C.

(b) *Syringe.*—10 mL with Luer tip.

(c) *Ultraviolet-visible (UV-Vis) spectrophotometer.*—Cary 118, or equivalent.

(d) *Isopropanol solutions.*—2.5, 5, 13, 20, and 50% in water.

(e) *FD&C reference standards.*—Division of Color Technology, Food and Drug Administration, Washington, DC. *Stock solution.*—100 mg/100 mL water. *Working solution.*—Dilute 1 mL stock solution to 100 mL with appropriate isopropanol solution to yield suitable spectra in 1 cm cells.

Samples

For ease of sample preparation, liquids or easily liquefied products are recommended for analysis. Acidic foods, i.e., beverages and beverage syrups, require no special preparation. Gelatin products should be dissolved in water (0.1 g in 3 mL), acidified (1 drop of 1% acetic acid), and filtered before analysis. Alcohol-containing materials (cologne) should be diluted 1:1 with water and acidified (1 drop of 1% acetic acid) before analysis to enhance dye retention (4).

Procedure

Remove plunger from 10 mL syringe, place long end of cartridge on Luer tip of syringe barrel, and pour 3 mL isopropanol into syringe barrel. Replace plunger, pump solution through cartridge, remove cartridge and discard eluate. Repeat, using 5 mL 1% acetic acid, followed by 2–3 mL filtered sample, and appropriate isopropanol solution according to scheme in Figure 1, collecting only colored portion. These steps may be repeated in succession when separating a mixture of colors requiring more than one isopropanol solution.

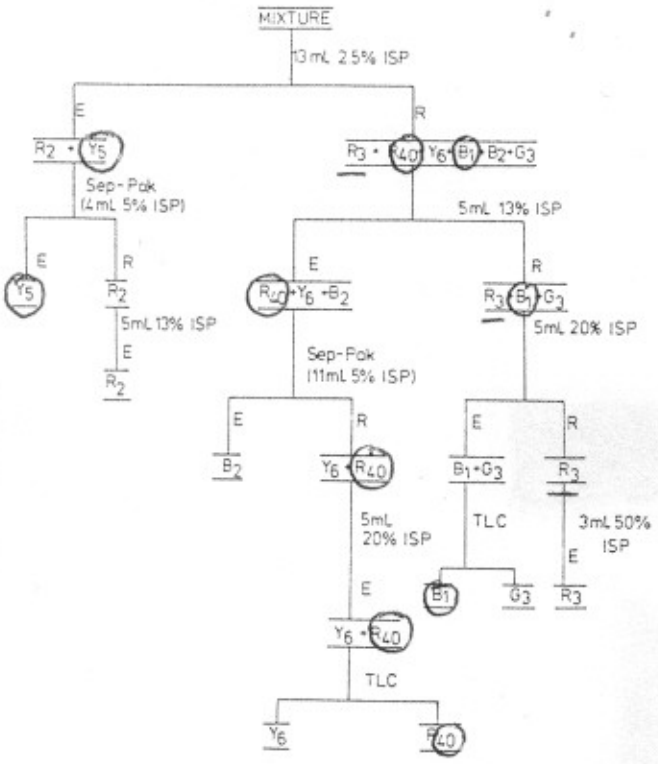


Figure 1. Color mixture schematic. Isopropanol (ISP): E, elutes and R, retains. R_2, Red No. 2; Y_5, Yellow No. 5; Y_6, Yellow No. 6; R_3, Red No. 3; R_{40}, Red No. 40; B_1, Blue No. 1; B_2, Blue No. 2; G_3, Green No. 3.

For those color combinations requiring a second reverse phase separation (C_{18} cartridge), evaporate eluate to ca 3 mL and load onto new cartridge prepared as above. Submerge collection tubes with sample extracts in water bath. Prepare control tubes marked at 3 mL level and filled with appropriate isopropanol solution that duplicates sample volume. Heat to boiling until desired concentration level is reached.

Confirm identity of separated colors by UV-Vis spectrophotometry, using combination of neutral-acid-basic spectra (Figure 2).

Separate FD&C Yellow No. 6/Red No. 40 and FD&C Blue No. 1/Green No. 3 by TLC (6), using silica gel G plates and *n*-butanol–methyl ethyl ketone–ammonium hydroxide–water (5 + 3 + 1 + 1). Streak 3 cm band of mixture of interest along base of TLC plate and dry. Spot appropriate reference standards at spaced intervals over dried sample streak, dry, and develop ca 10 cm. One common band develops at R_f of corresponding reference standard. Depending on colors in sample, it is usually unnecessary to follow entire scheme.

Ten commercial products (cologne, pudding mix, 2 gums, 4 candies, 2 syrups) (Table 1) were analyzed by using the technique described above. To extract the colorings, the syrups, gums, and candies were diluted, washed, or dissolved in water. The cologne was evaporated to near dryness before addition of water. All solutions were filtered through paper (Whatman 2V folded) and 2–3 mL was transferred to the cartridges. The eluates were transferred to suitable spectrophotometric cells and scans were obtained from 750 to 350 nm. All UV-Vis spectra that were obtained matched standard reference spectra.

Results and Discussion

The current method (1) used for the separation of the FD&C colors in foods requires several time-consuming steps. For products containing FD&C Red No. 3 and other colors, Procedure III (1) may require 4–5 h. The major time-consuming

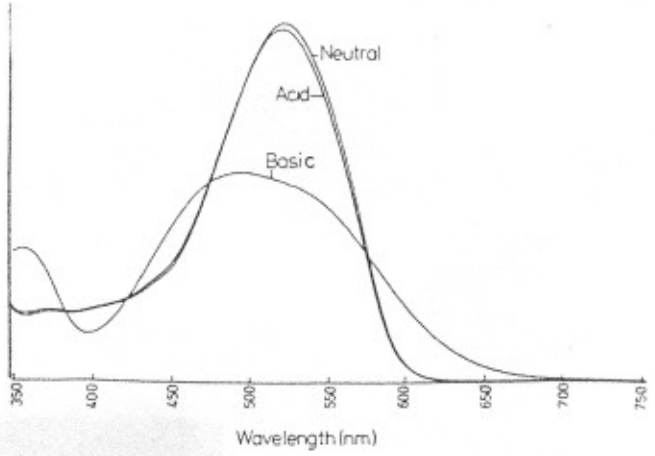


Figure 2. Spectra of FD&C Red No. 2 in 13% isopropanol.

step is the separation of the colors on a Solka-Floc column. By comparison, the procedure based on reverse phase C_{18} cartridge chromatography takes a maximum of 1 h. In many cases, when it becomes obvious that only one color is present, the procedure may require only 15 min.

The elution scheme was developed using 10 ppm concentrations of each of the 8 FD&C color additives in water. A sequence of solvent mixtures was used to selectively retain or elute the various color additives. Each successive solvent change represented a change in solvent strength beginning with the weaker solvent used to load the sample onto the cartridge. These different solutions were chosen so that particular colors in the sample would either elute rapidly with 2–7 mL solvent (e.g., FD&C Yellow No. 5, FD&C Green No. 3), or be strongly retained by the packing (e.g., FD&C Red No. 3). FD&C Red No. 2 is the only red color examined that eluted with 2.5% isopropanol, which accounts for the selection of that concentration. Sugars and flavorings are not retained by the cartridge but are flushed through upon addition of the dissolved sample.

The elution patterns may be affected by some product excipients, but if no interferences are present all analyses proceed as outlined. In lilac candy pops the FD&C Blue No. 2 eluted in 20% isopropanol instead of 13% isopropanol. We do not have an explanation for this observation. When this problem is encountered, however, the eluate is collected, corresponding reference standards are prepared in the same strength isopropanol solution, and UV-Vis spectra are obtained.

A tally was made of the most common FD&C single colors and color combinations found during routine sample analyses over a 2-year period. The single FD&C colors in descending order of occurrence were: Red No. 40, Red No. 3, Yellow No. 5, Red No. 2, Yellow No. 6, and Blue No. 1. FD&C Green No. 3 and Blue No. 2 were not found. The 5 most common FD&C color combinations were: *(1)* Yellow Nos. 5 and 6; *(2)* Yellow No. 5 and Blue No. 1; *(3)* Yellow Nos. 5 and 6, Blue No. 1, and Red No. 3; *(4)* Red No. 3 and Blue No. 1; or Blue No. 1, Yellow Nos. 5 and 6, and Red No. 40; and *(5)* Red No. 40 and Blue No. 1.

This tally was based on the number of times these colors appeared in 203 samples of products analyzed. All of the 7 permitted FD&C colors and the recently banned FD&C Red No. 2 are not likely to be found in combination at any one time. Therefore, the color mixture schematic provides a useful sequence of analysis for screening the colors.

The results of a number of color determinations are presented in Table 1. These data demonstrate the generality of

Table 1. Determination of colors in various products

Product	Product color	Isopropanol, %	FD&C colors detected
Rose syrup	red	2.5	Red No. 2
Rose syrup	red	2.5/13	Red No. 40
Gum balls	green	5	Yellow No. 5
		20	Blue No. 1
Gum balls	red-orange	13	Yellow No. 6
		50	Red No. 3
Candy pops (assorted)	lilac	20	Blue No. 2
		50	Red No. 3
	yellow-pink	5	Yellow No. 5
		13/50	Red No. 3
Strawberry milkshake candy	red & white	2.5/13	Red No. 3
		50	
Fish jelly candy	yellow	2.5/5	Yellow No. 5
Mice jelly candy	orange	2.5/5/13	Yellow No. 6
Pudding mix	white	2.5/13 *	Red No. 40
Florida water (cologne)	blue-green	13/50	unidentified yellow Blue No. 3

the method as a significant time-saving device in color analysis. With this system, FD&C Red Nos. 3 and 40 are easily separated. Three products, rose syrup (containing Red No. 2), pudding mix (containing Red No. 40), and strawberry milkshake candy (containing Red No. 3), demonstrate the different elution patterns of the 3 red colors: 2.5% isopropanol for Red No. 2, 13% isopropanol for Red No. 40, and 50% isopropanol for Red No. 3.

This method has been applied successfully to a variety of products and is being routinely used to rapidly screen many products received for color additive analysis in the New York Regional Laboratory.

Acknowledgment

The author thanks James F. Lawrence of the Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada, whose suggestions greatly improved this paper.

REFERENCES

(1) Graichen, C., & Molitor, J. C. (1963) *J. Assoc. Off. Agric. Chem.* 46, 1022–1029
(2) Publication B-23 (Sept. 1982) Waters Associates, Milford, MA, 12 pp
(3) Young, M.L. (1982) *Laboratory Information Bulletin No. 2610*, Food and Drug Administration, New York, NY
(4) Publication S-85 (April 1979) Waters Associates, Milford, MA, 4 pp
(5) McKone, H. T., & Ivie, K. (1980) *J. Chem. Educ.* 57, 321–322
(6) Graham, R. J. T., & Nya, A. E. (1969) in *Fifth International Symposium on Chromatography and Electrophoresis*, P. de Moerlosse (Ed.), Humphrey Science Publishers Inc., Ann Arbor, MI, 5, 486–490

Opmerkingen.

a) Het is duidelijk hiervoor wateroplosbare kleurstoffen gebruikt zijn. Deze lossen bij voorkeur in polaire oplosmiddelen op.

b) Rood hier lijkt het meest op Red#40 .

c) Alle drie de blauwe kleurstoffen vertonen het zelfde oplos gedrag.

Ook was de intensiteit die bij het extraheren verkregen wordt goed. Het ziet er naar uit dat al deze kleurstoffen zeer geschikt zijn als monster.

Adsorptie proeven.

Tabel 4: De adsorptie eigenschappen van crêpe papier kleurstoffen.

Kleurstof	Elutie % methanol	Opmerkingen
Blauw	70%	
Rood	15	
Geel	50	
Groen	15	Gele fractie
	50	Blauwe fractie
Bruin	15	Rode fractie
	30-50	Bruine fractie
Zwart	70	Blauw groene fractie
		Niet schoon, blijft zwart!
Paars	50-70	Roze fluorescerend

Opmerkingen:

a)Deze blauwe kleurstof is toch anders dan bij de Flavor-Aid kleurstoffen. Deze elueert bij een veel hoger gehalte methanol (70% tov 50%).

b)Rood is ook anders, elueert bij een veel lager gehalte methanol (15% tov 30%)

c)Geel is ook anders, elueert bij een veel hoger gehalte methanol (50% tov 15%).

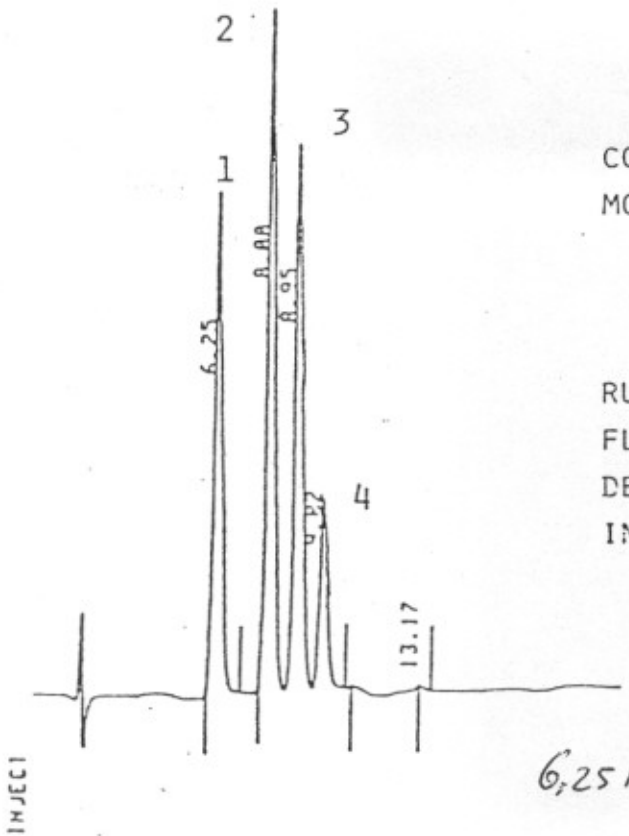
De combinaties geven nieuwe mogelijkheden voor experimenten.

d)Groen en bruin zijn samengestelde kleuren. Het lijkt er op dat er bij groen niet dezelfde blauwe kleur gebruikt is.

e)Het voordeel van crêpe papier is dat er niet gefiltreerd hoeft te worden.

SYNTHETIC DYES IN FOOD

YELLOW/ORANGE - STANDARDS



6,25 MIN

COLUMN:	RAD PAK C18
MOBIL PHASE:	A - 0,005 M TBA
	B - ACETONITRILE
	30% B → 50% B
	CURVE 6
RUN TIME:	20 MIN.
FLOW:	2,00 ML/MIN.
DETECTION:	M450/450 NM/0,04 AUFS
INJECTION:	20 UL

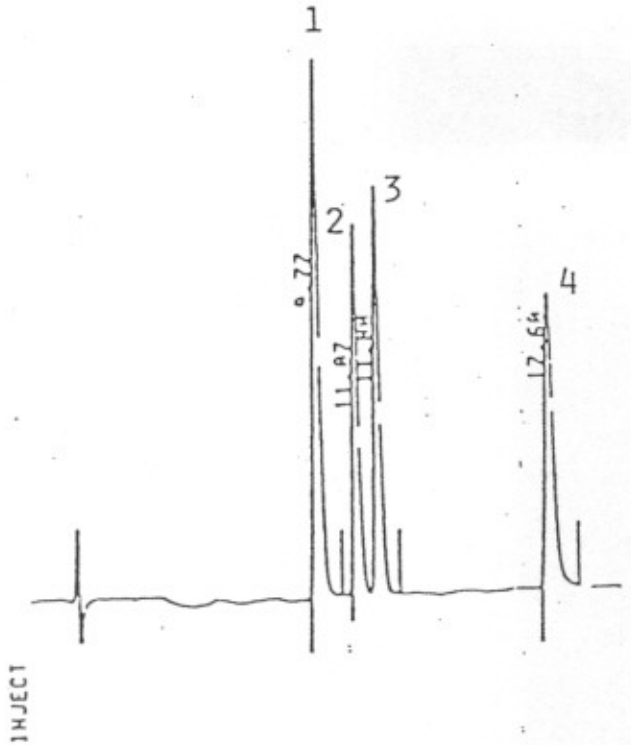
1. QUINOLINE YELLOW 1
2. SUNSET YELLOW FCF
3. TARTRAZINE
4. QUINOLINE YELLOW 2

QUINOLINE YELLOW 20 MG/L - SUNSET YELLOW FCF 30 MG/L - TARTRAZINE 16 MG/L

E.H.-S. / M.B. - DENMARK

SYNTHETIC DYES IN FOOD

RED - STANDARDS



COLUMN: RAD PAK C18
MOBIL PHASE: A - 0,005M TBA
B - ACETONITRILE
30% B → 50 % B CURVE 6
RUN TIME: 20 MIN.
FLOW: 2,00 ML/MIN.
DETECTION: M450/520 NM/0,04 AUFS
INJECTION: 20 UL

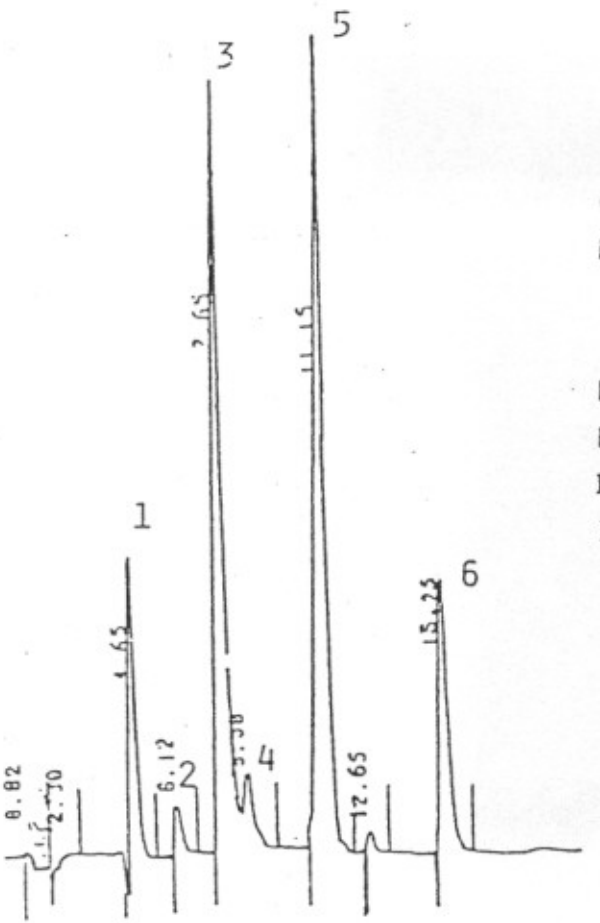
1. AMARANTH
2. PONCEAN 4R
3. AZORUBIN
4. ERYTHROSINE

AMARANTH 8 MG/L - PONCEAU 4R 9 MG/L
- AZORUBIN 8 MG/L - ERYTHROSINE 8 MG/L

E.H.-J. /M.B. - DENMARK

SYNTETIC DYES IN FOOD

GREEN/BLUE - STANDARDS



COLUMN: RAD PAK C18
MOBILE PHASE: A-0,005 M TBA
B-ACETONITRILE
30% B → 50% B CURVE 6
RUN TIME: 20 MIN.
FLOW: 2,00 ML/MIN.
DETECTION: M450/620 NM/0,04 AUFS
INJECTION: 20 UL

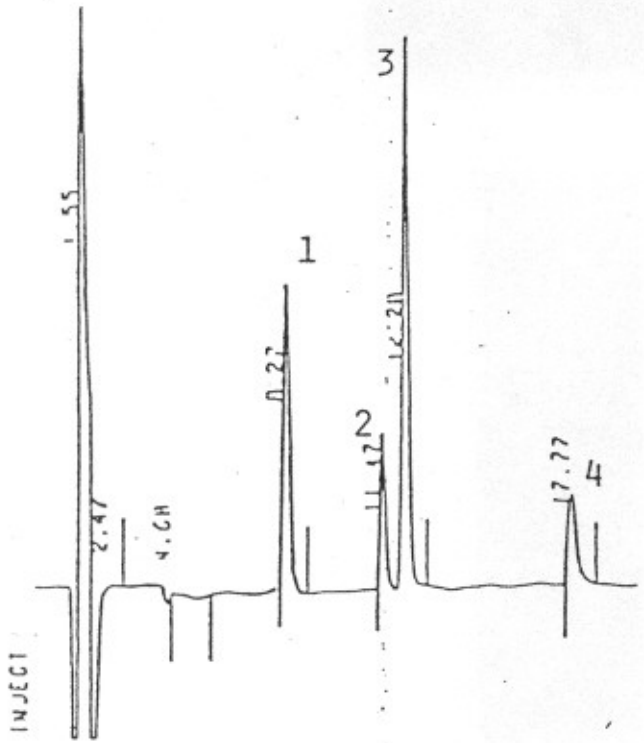
1. INDIGOTIN I 1
2. GREEN S 1
3. GREEN S 2
4. INDIGOTIN I 2
11,15 MIN 5. BRILLIANT BLUE FCF/BLUE No:
6. PATENT BLUE V

INDIGOTIN I 11 MG/L - GREENS 11 MG/L - BRILLIANT BLUE FCF 10 MG/L - PATENT BLUE V 4 MG/L

E.H-J./M.B. - DEC.

SYNTETIC DYES IN FOOD

CANDY - SAMPLE



COLUMN:	RAD PAK C18
MOBILE PHASE:	ACETONITRILE/ 0,005 M TBA LIN. GRAD.
RUN TIME:	20 MIN.
FLOW:	2,0 ML/MIN.
DETECTION:	M450/520 NM/0,0
INJECTION:	20 UL

1. SUNSET YELLOW FCF
2. PONCEAU 4R
3. AZORUBIN
4. ERYTHROSINE

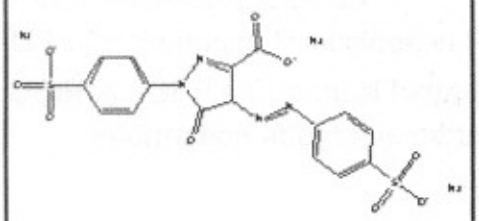
RED DROPS CLEANUP PROCEDURE I

E.H.-J./M.B.- DENMI

Staalmeesters Consultancy • Nieuweweg 51 • 4631 TD Hoogerheide • Tel. 06-20547941 • Fax 0164-660046
Bankrekening Nr: 1229.78.366 • K.v.K. Nr: 20105025 • E-mail: steelmasters@hetnet.nl
BTW No: NL0728.15.486.B01

Bijlage 5

FD&C Yellow No. 5 [1934-21-0]
Synonyms: 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-1H-Pyrazole-3-carboxylic acid, trisodium salt; Acid Yellow 23; Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-1H-pyrazole-3-carboxylic acid, trisodium salt; C.I. 19140; C.I. Acid Yellow 23; FD&C Yellow No. 5; Filter Yellow; Food Yellow No.4; Pyrazole-3-carboxylic acid, 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-, trisodium salt; Tartrazine; Tartrazine C; Tartrazine C.I.19140; Trisodium 1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-5-pyrazolone-3- carboxylate; Trisodium 1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-1H-pyrazole-3-carboxylate;



Formula	$C_{16}H_9N_4Na_3O_9S_2$	Molecular Weight	534.35781
CAS RN	1934-21-0	Melting Point (°C)	
ACX Number	X1005210-7	Boiling Point (°C)	
Density		Vapor Density	
Refractive Index		Vapor Pressure	
Evaporation Rate		Water Solubility	
Flash Point (°C)		EPA Code	
DOT Number		RTECS	UQ6400000
Comments	HYGROSCOPIC.		

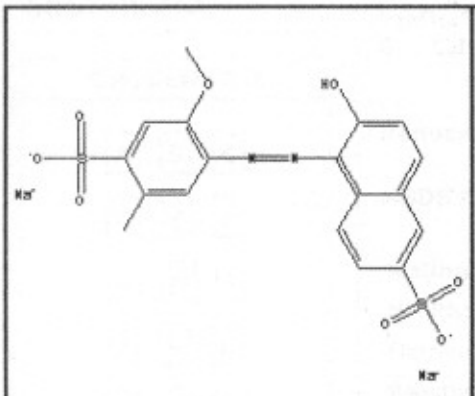
More information about the chemical is available in these categories:

Health | Misc | Pesticides/Herbicides | Physical Properties | Regulations | Trading

Health (4)
8(e) TRIAGE Chemical Studies Database

FD&C Red No. 40

Synonyms: Allura Red AC; C.I. 16035; FD&C Red No. 40;



Tools

VIEW CHEMDRAW STRUCT

VIEW CHEM3D MODEL

CAS RN Lookup

THE MERCK INDEX

NCI DATABASE

OpenChem

VIEW LINKS

ADD COMPOUND

ADD/CHANGE PROPERTY

ADD LINK

Formula	$C_{18}H_{14}N_2Na_2O_8S_2$	Molecular Weight	496.41674
CAS RN		Melting Point (°C)	
ACX Number	X1011809-1	Boiling Point (°C)	
Density		Vapor Density	
Refractive Index		Vapor Pressure	
Evaporation Rate		Water Solubility	
Flash Point (°C)		EPA Code	
DOT Number		RTECS	
Comments			

More information about the chemical is available in these categories:

Health (1)

Center for Food Safety & Applied Nutrition

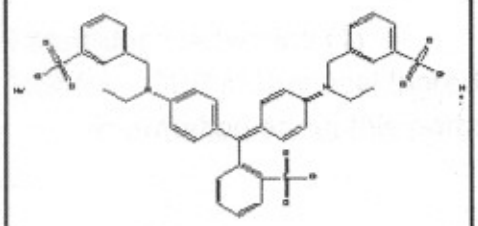
Information about this particular compound

Misc (1)

UWI-Mona Chemistry Lectures

Information about this particular compound

FD&C Blue No. 1 [3844-45-9]
Synonyms: Acid Blue 9, disodium salt; Alphazurine FG; Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo-, inner salt, disodium salt; Bis[4-(N-ethyl-N-3-sulfophenylmethyl)aminophenyl]-2-sulfophenylmethylium disodium salt; Disodium bis[4-(N-ethyl-N-3-sulfonatophenylmethyl)aminophenyl]-2- sulfonatophenylmethylium; Brilliant blue FCF; Brilliant blue FCF, disodium salt; C.I. 42090; Erioglaucine; Erioglaucine disodium salt; Erioglaucine disodium salt (C.I. 42090); FD&C Blue No. 1; Food Blue 2; Food Blue No.1;



Formula	$C_{37}H_{34}N_2Na_2O_9S_3$	Molecular Weight	792.84314
CAS RN	3844-45-9	Melting Point (°C)	283
ACX Number	X1005351-3	Boiling Point (°C)	
Density		Vapor Density	
Refractive Index		Vapor Pressure	
Evaporation Rate		Water Solubility	
Flash Point (°C)		EPA Code	
DOT Number		RTECS	BQ4725000
Comments	Coloring.		

More information about the chemical is available in these categories:
Health Misc Pesticides/Herbicides Regulations Trading

Health (5)
8(e) TRIAGE Chemical Studies Database