

COMMUNICATION

IMPORTANT INFORMATION FOR READERS OF HIGH-PERFORMANCE
LIQUID CHROMATOGRAPHY LITERATURE

V. Das Gupta, Yashoda Pramdar and Jagdish Parasrampuriah*

Department of Pharmaceutics
College of Pharmacy
University of Houston
Houston, TX 77030
and

*Abbott Laboratories
Abbott Park, IL 60064

ABSTRACT

Important information for developing an internal standard for high-performance liquid chromatography has been presented in a tabular form. If acetonitrile is substituted for methanol, the column lives are longer and costly repairs of the chromatograph are not needed that often. Approximately 1% of acetonitrile can be substituted for 2% of methanol.

BACKGROUND

When developing a new HPLC assay method for a drug, it is often necessary to find an appropriate internal standard. The internal standard is required for accuracy of the results. The authors of this report have prepared Table 1, which lists chromatographic conditions of various compounds, based on their experience in the laboratory. This table has been found to be very useful for the purpose of developing an internal standard. The table lists the

TABLE 1
CHROMATOGRAPHIC CONDITIONS WITH
AN INCREASING STRENGTH OF THE MOBILE PHASE

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Metronidazole (Phenylpropanolamine HCl)	254	200 1400	0.02M potassium phosphate, pH = 4.2, phenyl column
5-Flucytosine (5-Aminouracil)	300	100 12	0.05M phosphate, pH = 4.5, C ₁₈ column
Carbenicillin	230	600	.02M ammonium acetate, phenyl column
Moxalactam	254	80	.02M ammonium acetate, pH = 5.8, phenyl column
Tobramycin	230	100	.02M ammonium acetate, phenyl column
Ticarcillin	245	600	.01M ammonium acetate, phenyl column
Hydralazine HCl (Phenylpropanolamine HCl)	256	30 1600	2% MeOH, .1% HAc, 0.015M phos- phate, C ₁₈ column
Phenylephrine HCl (Acetaminophen)	271	225 120	2% ACN, .02M phosphate, pH = 5.9-6.1, C ₁₈ column
Cefadroxil (Ceftazidime)	254	67 133	2% ACN, 0.01M ammonium ace- tate, C ₁₈ column
Pseudoephedrine (Salicylamide)	257	30 400	4% MeOH, 1% HAc, .02M ammonium acetate, pH = 3.8, C ₁₈ column
Acetaminophen (Salicylamide)	257	16 400	5.5% MeOH, 1% HAc, .02M ammo- nium acetate, pH = 3.8, C ₁₈ column
Cefsulodin	280	1000	3.5% MeOH, 1.5% ACN, .02M ammonium acetate, pH = 4.1, phenyl column
Hydrochlorthiazide (Salicylamide)	273	40 300	7% MeOH, .5% HAc, .02M ammoni- um acetate, C ₁₈ column
Morphine	254	300	7% MeOH, 0.1M phosphate, C ₁₈ column

TABLE 1 (continued)

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Acyclovir (Salicylic acid)	252	7 240	3% ACN, 0.01M phosphate, C_{18} column
Isoetharine (Metaproterenol)	278	100 100	3.5% ACN, 1% HAc, C_{18} column
Levodopa Dopamine (Carbidopa)	280	50 50 50	7% MeOH, 2% HAc, 0.005M heptanesulfonic acid, C_{18} column
Terbutaline SO_4 (Salicylic acid)	278	200 50	8% MeOH, .02M phosphate, pH = 3.6, phenyl column
Potassium Guaiacolsulfonate	254	100	8.5% MeOH, .005M dibasic ammonium phosphate pH = 7.75, C_{18} column
Benzoic Acid	254	300	10% MeOH, .02M phosphate, pH = 6.2, C_{18} column
Phenol	254	100	10% MeOH, .02M ammonium bicarbonate, pH = 7.67, C_{18} column
Ceftazidime (Cephalexin)	240	35 105	6% ACN, .01M ammonium acetate, phenyl column
Cefotetan Disodium (Cephalexin)	262	30 40	6% ACN, 0.01M phosphate, C_{18} column
Codeine PO_4 Guaifenesin Pheniramine Maleate Phenylpropanolamine HCl Pyrilamine Maleate	254	100 100 1000 150 200	13% MeOH, .05M phosphate, C_{18} column
Ampicillin Na	240	500	7% ACN, .01M phosphate, pH = 4.1, C_{18} column
Famotidine (Sulfamerazine)	268	40 28	12% MeOH, 2% ACN, .1% HAc, .01M phosphate, C_{18} column
Baclofen (Sulfamerazine)	266	400 20	12% MeOH, 2% ACN, .02M phosphate, C_{18} column

(continued)

TABLE 1 (continued)

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Acetazolamide (Sulfamerazine)	265	15 15	12% MeOH, 2% ACN, .02M phosphate, C_{18} column
Sulfacetamide	257	32	16% MeOH, 0.02M phosphate, C_{18} column
Sulfamerazine		32	
Sulfadiazine		32	
Sulfamethazine		32	
Vancomycin	282	500	8.5% ACN, .02M phosphate, pH = 3.5, phenyl column
Cephalexin (Cefazolin)	260	40 60	9% ACN, .01M ammonium acetate, phenyl column
Cefotaxime Sodium	254	100	18% MeOH, .02M ammonium acetate, phenyl column
Cefuroxime (Cefazolin)	274	250 750	18.5% MeOH, .02M ammonium acetate, pH = 6.7, phenyl column
Hydrocortisone	254	20	20% MeOH, .02M phosphate, cyano column
Salicylic Acid	254	10	20% MeOH, .01M phosphate, pH = 2.3, C_{18} column
Acetaminophen		50	
Aspirin		150	
Salicylamide		150	
Caffeine		25	
Codeine PO_4		100	
Phenacetin		40	
Salicylic Acid	300	25	20% MeOH, 0.01M phosphate, pH = 2.3, C_{18} column
Cephadrine (Cefazolin)	260	80 120	8% MeOH, 6% ACN, .1% HAc, .01M ammonium acetate, phenyl column
Cefoxitin	254	60	20% MeOH, .02M ammonium acetate, phenyl column
Ranitidine (Caffeine)	262	60 30	10% MeOH, 7% ACN, .01M phosphate, pH = 5.8, C_{18} column

TABLE 1 (continued)

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Furosemide	254	40	25% MeOH, .01M dibasic ammonium phosphate, C ₁₈ column
Cocaine HCl (Ethyl p-aminobenzoate)	275	200 5	25% MeOH, .02M ammonium acetate, pH = 7, cyano column
Chloroquine PO ₄ (Dobutamine HCl)	257	50 400	26% MeOH, .02M phosphate, pH = 4.3, phenyl column
Phenylephrine HCl	254	50	13% ACN, 1.8% HAc, .005M
Phenylpropanolamine HCl		50	1-heptanesulfonic acid sodium
Brompheniramine Maleate		40	salt, pH = 2.6, cyano column
(Sodium Benzoate)		50	
Cefazolin	254	60	30% MeOH, .01M ammonium acetate, phenyl column
Cephalothin	254	50	30% MeOH, .01M ammonium acetate, phenyl column
Cefoxitin (Cefazolin)	262	50 30	15% ACN, .02M phosphate, pH = 4.0, C ₁₈ column
Haloperidol (Medroxyprogesterone)	254	40 40	30% MeOH, .02M phosphate, cyano column
Disulfiram (Hydrocortisone)	254	100 40	30% MeOH, .01M phosphate, cyano column
Progesterone	254	40	30% MeOH, .02M phosphate, cyano column
Medroxyprogesterone Acetate		40	
(Hydrocortisone)		20	
Triamcinolone Acetonide (Hydrocortisone)	254	80 32	32% ACN, .02M phosphate, pH = 4.2, C ₁₈ column
Phenobarbital (Mezlocillin)	230	150 600	35% MeOH, .02M ammonium acetate, phenyl column
Cefoperazone (Oxacillin)	240	12 24	18% ACN, .01M ammonium acetate, phenyl column
Piperacillin Sodium	230	100	38% MeOH, .02M ammonium acetate, phenyl column

(continued)

TABLE 1 (continued)

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Penicillin G Potassium	240	400	20% ACN, .01M phosphate, pH = 4.2, C_{18} column
Cefamandole	254	800	40% MeOH, .02M ammonium acetate, phenyl column
Ciprofloxacin HCl (Cephalothin)	276	12	15% ACN, 12% MeOH, 0.3% HAc, 0.01M phosphate, C_{18} column
Norfloxacin (Cephalothin)		225	
		8	
		180	
Dobutamine HCl (Methapyrilene HCl)	278	100	20% ACN, .02M phosphate, .3% HAc, pH = 3.6, phenyl column
		400	
Hydroxyprogesterone Caproate (17-Hydroxyprogesterone)	254	60	40% MeOH, .02M phosphate, cyano column
		20	
Spirolactone	254	50	40% EtOH, .02M phosphate, cyano column
Antipyrine (Benzocaine)	254	100	40% MeOH, .02M phosphate, C_{18} column
		50	
Chlorpheniramine Maleate (Phenyltoloxamine Citrate)	262	60	42% MeOH, 1% HAc, 1% ammonium formate, pH = 4.3, C_{18} column
		120	
Mezlocillin Sodium	230	35	42% MeOH, .02M ammonium acetate, phenyl column
Propranolol HCl (Verapamil HCl)	270	100	43% MeOH, .5% HAc, .02M ammonium formate, C_{18} column
		300	
Chlorpropamide (Tolbutamide)	232	50	45% MeOH, .02M ammonium acetate, .5% HAc, phenyl column
		100	
Ethacrynic Acid	254	50	45% MeOH, .01M ammonium phosphate, C_{18} column
Glipizide (Hydrocortisone)	232	25	24% ACN, .02M ammonium acetate, phenyl column
		26	
Nafcillin	280	80	50% MeOH, .01M ammonium acetate, phenyl column
Dexamethasone	254	35	50% MeOH, .01M phosphate, C_{18} column

TABLE 1 (continued)

Drug (Internal Standard)	Wavelength (nm)	Conc. ($\mu\text{g/ml}$)	Mobile Phase and the Column Used
Prednisolone	254	50	50% MeOH, .01M phosphate, C ₁₈ column
Primaquine PO ₄ (Dextromethorphan)	278	100 360	29% ACN, .5% HAc, 0.01M phos- phate, phenyl column
Glyburide (Hydrocortisone)	232	50 26	30% ACN, .02M ammonium ace- tate, phenyl column
Spironolactone (Methyl Testosterone)	254	50 60	63% MeOH, 0.01M phosphate, C ₁₈ column
Spironolactone (Methyl Testosterone)	254	80 24	39% ACN, 0.01M phosphate, phenyl column
Hydrocortisone	254	40	32% ACN, .02M phosphate, C ₁₈ column
Fluoxymesterone (Hydrocortisone)	254	80 40	32% ACN, .01M phosphate, C ₁₈ column
Verapamil HCl (Dextromethorphan HBr)	278	250 200	38% ACN, .02M phosphate, phenyl column
Procainamide HCl (Methapyrilene)	280	15 200	40% ACN, .02M ammonium ace- tate, pH = 7, cyano column
Thiopental	296	5	54% ACN, .01M ammonium ace- tate, C ₁₈ column
Lignocaine (Verapamil)	261	800 256	54% ACN, .01M phosphate, pH = 7.5, C ₁₈ column
Meperidine HCl (Hydroxyprogesterone Caproate)	232	20 30	60% ACN, .02M ammonium ace- tate, C ₁₈ column
Benzoyl Peroxide (Hydroxyprogesterone Caproate)	254	40 80	60% ACN, .8% HAc, C ₁₈ column

drugs based on the mobile phase required (with an increasing strength). The wavelengths have been presented since the drug may not absorb light at higher wavelengths (esp. above 300 nm). The other information in Table 1 has been presented to make an intelligent decision for the selection of a compound which will most likely work as an internal standard.

Another useful hint, for every 2% methanol, ~1% of acetonitrile is usually a good substitute for similar results. With acetonitrile, the column lives are longer than with methanol since pressure is much lower. Furthermore, the chromatograph will not need repairs as often as with methanol.