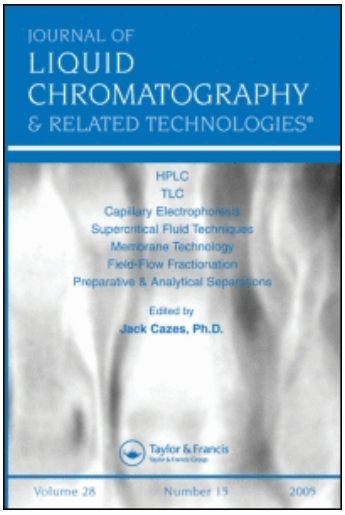


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Recent Advances in HPLC Analysis of Analgesics

Terry D. Wilson[a]
[a] Analytical Chemistry Department, Sterling-Winthrop Research Institute Rensselaer, New York

To cite this Article Wilson, Terry D.(1986) 'Recent Advances in HPLC Analysis of Analgesics', Journal of Liquid Chromatography & Related Technologies, 9: 11, 2309 — 2409
To link to this Article: DOI: 10.1080/01483918608076873
URL: http://dx.doi.org/10.1080/01483918608076873

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(11), 2309-2409 (1986)

RECENT ADVANCES IN HPLC ANALYSIS OF ANALGESICS

Terry D. Wilson
Analytical Chemistry Department
Sterling-Winthrop Research Institute
Rensselaer, New York 12144

INTRODUCTION

Recent developments in HPLC analysis of analgesics have been rapid, paralleling the growth of chromatographic sciences as a whole. Although analgesics have been used for hundreds of years and chromatographic separations have been carried out on them for decades, liquid chromatographic analysis of analgesics has advanced most rapidly in the past 5-7 years. The present review will focus on applications of HPLC to the analysis of commercial analgesics in this time period with emphasis on novel developments and applications associated with the improved accuracy, sensitivity and specificity inherent in current LC systems.

Previous reviews have included analysis of analgesics by liquid chromatography as a class of abused

 0148-3919/86/0911-2309$3.50/0

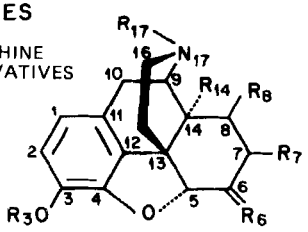
drugs in forensic science. Fishbein in a comprehensive review emphasized metabolic studies on narcotics and analgesics by GLC, TLC, HPLC and GC/MS (1). This included work published predominantly prior to 1980 with more LC coverage given to acetaminophen, phenacetin and the salicylates. Similarly Gough and Baker reviewed chromatographic methods including TLC, GLC and HPLC for narcotics as a class of drugs of abuse (2). Lurie, more recently reviewed the use of HPLC in forensic chemistry (3). Specifically he discussed the application of bonded-phase separations for drugs of abuse. The present review will deal only with the HPLC analysis of analgesics which have appeared in print roughly between 1980 and 1985 and include older material not covered by previous reviews. In this case analgesics will be classified according to Willette (4) into two groups: the opiates and the anti-inflammatory analgesics. The latter group will be subdivided into salicylic acid derivatives, p-aminophenol derivatives, arylacetic acids and pyrazole derivatives. Further each of these groups will be subdivided into HPLC studies related to metabolism, dosage forms and other studies where available. The reader is referred to references 1-4 for descriptions of history, pharmacology, structure-activity relationships and metabolic status of these compounds current at publication.

I. OPIATES

The opiates shown in Figure 1a-f include compounds from the morphine, oripavine, morphinan, benzomorphan, piperidine and phenylpropylamine structural groups. Similarities in structure are apparent and their development has been covered elsewhere.

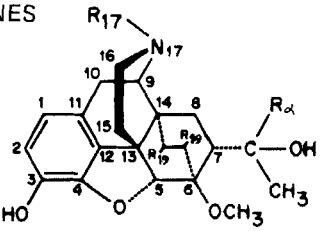
I) OPIATES

a) MORPHINE DERIVATIVES



	R_3	R_6	R_7 R_8	R_{14}	R_{17}
MORPHINE	$-H$			$-H$	$-CH_3$
CODEINE	$-CH_3$			$-H$	$-CH_3$
DIHYDROCODEINE	$-CH_3$			$-H$	$-CH_3$
HEROIN				$-H$	$-CH_3$
OXYCODONE	$-CH_3$			$-OH$	$-CH_3$
NALBUPHINE	$-H$			$-OH$	$-CH_2$ 
OXYMORPHONE	$-H$			$-OH$	$-CH_3$
HYDROMORPHONE	$-H$			$-H$	$-CH_3$
HYDROCODONE	$-CH_3$			$-H$	$-CH_3$

Ib) ORIPAVINES



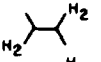
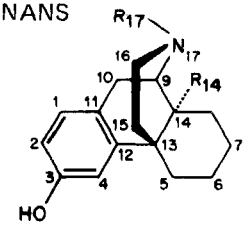
	R_α	R_{17}	R_{18} R_{19}
BUPRENORPHINE	$-C(CH_3)_3$	$-CH_2$	
ETORPHINE	$-CH_2CH_2CH_3$	$-CH_3$	

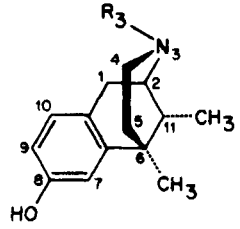
FIGURE 1. Structure of opiates. (continued)

Ic) MORPHINANS



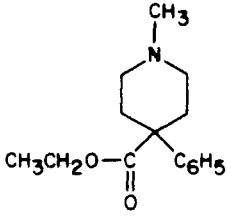
	R_{14}	R_{17}
LEVORPHANOL	—H	$-CH_3$
BUTORPHANOL	—OH	$-CH_2-$ 

Id) BENZOMORPHANS

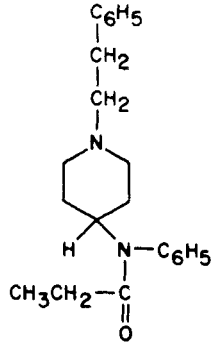


	R_3
PENTAZOCINE	$-CH_2-CH=C(CH_3)-CH_3$
CYCLAZOCINE	$-CH_2-$
PHENAZOCINE	$-CH_2CH_2-C_6H_5$

Ie) PIPERIDINE DERIVATIVES



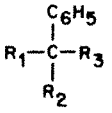
MEPERIDINE



FENTANYL

FIGURE 1 (continued)

f) PHENYLPROPYLAMINE DERIVATIVES



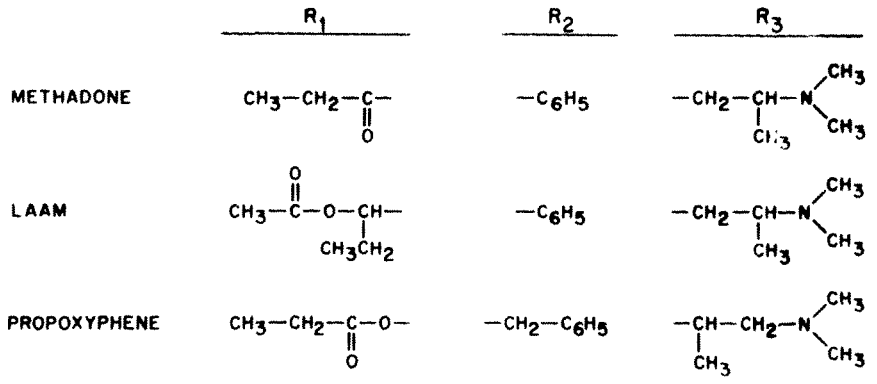


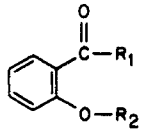
FIGURE 1 (continued)

Biological Studies

Many studies on morphine as the prototype opiate have been conducted in humans using HPLC. These have included pharmacokinetics measurements with detection predominantly by amperometric means utilizing the low oxidation potential of the 3-phenolic group. White used a flow cell constructed with a modified thin-layer wall-jet arrangement with a glassy carbon working electrode (5). While an 81% extraction effeciency was reported no interference was seen from other analgesics aside from cyclazocine in this normal phase assay. The more common reversed-phase separation of morphine in human serum using amperometric detection was pioneered by Wallace and coworkers (6). A recovery of 79% was found for morphine with no interference

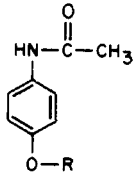
II ANTI-INFLAMMATORY ANALGESICS

a) Salicylic Acid Derivatives



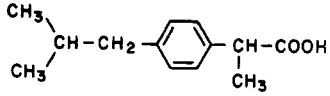
	R_1	R_2
SALICYLIC ACID	—OH	—H
ACETYLSALICYLIC ACID	—OH	—C(=O)—CH_3
SALICYLAMIDE	—NH_2	—H

b) p—Aminophenol Derivatives

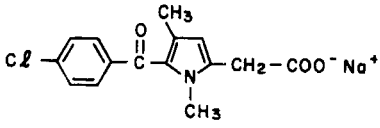


	R
ACETAMINOPHEN	—H
PHENACETIN	—CH_2CH_3

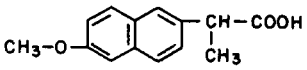
c) ARYLACETIC AND ARYLPROPIONIC ACIDS



IBUPROFEN



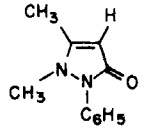
ZOMEPIRAC



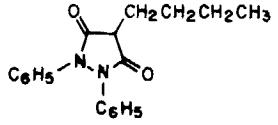
NAPROXEN

FIGURE 2. Structures of anti-inflammatory analgesics. (continued)

d) PYRAZOLE DERIVATIVES



ANTIPYRINE



PHENYLBUTAZONE

FIGURE 2 (continued)

from codeine, meperidine, methadone or pentazocine. Suitable chromatography was obtained by another group using ion-pairing reversed-phase LC for morphine in serum extracts (7). A 40° column temperature was necessary in the above study with similar overall recoveries found for morphine. Comparisons were made between the proposed method and previously published methods including fluorometry, RIA and GC with the conclusion that the HPLC method was simpler with increased recovery and required smaller sample volumes. Amperometric detection for the HPLC assay of plasma morphine was also compared to a GC assay where generally improved statistics for recovery were obtained in the former procedure with an overall recovery of 85% found (8). Electrochemical detection for morphine was also employed by Aitkenhead and coworkers with calculations of pharmacokinetic parameters in normal patients and ones with renal failure (9) and for a sustained release dosage form (10). Ion-pairing

was used in the reversed-phase method with 100.9% recovery reported for plasma standards in the first study. Another group used electrochemical and ultraviolet detectors in series in a study relating patient plasma morphine and diazepam levels to baroreflex control of heart rate (11). The electrochemical method for detection of morphine in plasma has met with such success that a general clinical procedure has been described using a reversed-phase separation with amperometry at 0.70v (12). Ultraviolet detection alone was used in a study by Svensson et al on human plasma and urine morphine levels (13). Sample preparation was simplified by use of a solid phase extraction which gave 84% and 90% recoveries for morphine and morphine-3-glucuronide respectively.

Studies on morphine disposition in animals have been carried out by HPLC using electrochemical, ultraviolet and fluorescence detection methods. Plasma and CSF levels of morphine have been determined in monkeys using electrochemical detection at a glassy carbon working electrode at 0.79v vs. Ag/AgCl. Extraction effeciencies were compared using a chloroform-isobutanol solvent mixture as opposed to an extraction column with 78.0 and 84.8% found for morphine respectively (14). A study on morphine levels in mouse blood also utilized EC detection at 0.725v in a reversed-phase system (15). A simple deproteinization step followed by centrifugation was used to give an improved detection limit. The same group also determined morphine in mouse brain using amperometric detection (16). Morphine in rat serum was assayed using HPLC with electrochemical detection and a chloroform extraction to give 75% recovery (17). A chloro-

form-isopropanol extraction scheme was used by other workers to determine morphine levels in fetal lamb plasma (18). An overall recovery of 92% for morphine was demonstrated with no interference from oxymorphone observed. Ultraviolet detection was used by Rane and coworkers who showed that the metabolite, morphine-3-glucuronide was present in monkey plasma at 8-11 times the morphine level immediately following i.v. administration (19). Fluorescence of underivatized morphine was measured in an HPLC assay to determine pharmacokinetics in rats in relation to the use of resuscitation fluids (20). An ethyl acetate-isoamyl alcohol solvent mixture gave an extraction effeciency from plasma of 88% at pH 7.4. The fluorescence of dansyl-derivatized morphine which was extracted from urine was measured in a normal phase system (21).

Codeine, the antitussive analgesic, differs from morphine only in the 3-position having a methoxy group in place of the hydroxyl of the latter. Not surprisingly similar HPLC systems and extraction schemes have been developed for the two drugs with several reports of their simultaneous determination in biological fluids. Ultraviolet detection at 230 nm was used in one study to determine morphine and codeine in urine (22). Sample preparation included adsorption on XAD-2 and chloroform-isobutanol extraction steps which gave recoveries of 64% for codeine and 38% for morphine. A normal phase method was developed using methadone as internal standard following a dichloromethane extraction to give 99.8% recovery of codeine (23). Codeine and morphine were extracted from serum and urine in another study which used a CN column in a reversed-phase mode (24). Analytes were detected by

UV at 210 nm with no interference found from hydromorphone or hydrocodone. The same authors measured codeine, morphine and norcodeine content of urine with the finding that the concentration of morphine, the metabolite, eventually surpassed codeine (25). Codeine has also been measured in plasma by a reversed-phase method using UV detection at 220 nm giving 98.4% recovery with preparation done on a C_{18} extraction column (26).

Codeine in biological fluid has been detected by means other than ultraviolet absorption. A fraction collecting approach was used by Nelson and coworkers to combine the selectivity of HPLC with the sensitivity of radioimmunoassay (RIA) and the less sensitive enzyme multiplied immunoassay techniques (EMIT) (27). This overcame the lack of specificity of the RIA and EMIT methods for codeine, morphine and their metabolites. Fluorescence detection was used for nonderivatized codeine which was extracted from plasma with hexane-dichloromethane (2:1) (28). The simple solvent extraction did not remove morphine from the plasma to permit its measurement with codeine. An amperometric determination of codeine in serum and urine has been carried out using an oxidation potential of 1.15 v (29). A Sephadex C-25 column was used here to clean up urine samples while an ethyl acetate extraction was successful for serum codeine.

Heroin metabolism has been studied by at least two groups using HPLC methadology. One developed a reversed-phase system to separate heroin from 6-O-acetylmorphine, morphine and normorphine (30). Detection was by UV at 254 nm although greater reliance was placed on liquid scintillation analysis of plasma pro-

tein binding, hydrolysis kinetics and red blood cell-plasma partitioning of heroin. Ultraviolet detection was also used by Inturrisi and coworkers on hydrolysis and pharmacokinetics of heroin in human and rat blood (31). A solvent extraction process with 1-α-acetylmethadol (LAAM) as internal standard afforded 93% recoveries of heroin, acetylmorphine and morphine.

Oxycodone was determined in plasma using reversed phase HPLC with amperometric detection at 1.20 v (32). A butyl chloride extraction gave 74% recovery for oxycodone and 80.2% for the internal standard, methadone. Dihydrocodeine pharmacokinetics have been examined using HPLC with UV detection at 282 nm (33). A butyl acetate plasma extract was acidified and injected into the reversed-phase system to show that renally impaired patients have slower absorption and elimination than normal patients.

Nalbuphine, an analgesic agonist/antagonist of the morphine class, can be detected easily by electrochemical means following HPLC because of the 3-phenolic hydroxyl group. As in the case of morphine but not codeine or oxycodone, oxidation potentials of only 0.75 v are required for this reaction. Lake and coworkers observed this behavior with a cyclic voltammetry study in determining plasma nalbuphine levels in cardiac surgical patients (34). A one step ethyl acetate-2-propanol extraction was used following deproteination to give 94% recovery for nalbuphine. A modified extraction method was described by others to overcome certain difficulties in the above method (35). An extraction mixture of ethyl acetate-toluene-2-propanol with a back-extraction step along with careful control of pH were required here. Similarly an

LC system with an autosampler and electrochemical detection at 0.95 v was used to measure nalbuphine in plasma (36). Recoveries over 90% were reported which permitted the calculation of elimination parameters and bioavailability.

Analgesics of the morphinan class, levorphanol and butorphanol have been determined in biological fluids by HPLC. A lack of sensitivity was reported by de Silva in measuring levorphanol, a metabolite of 3-methoxy-N-methylmorphinan in plasma by HPLC with ultraviolet detection (37). The method which included a pH 11 heptane extraction was sensitive only to 250 ng/mL levorphanol with no improvement found using fluorescence detection. Lucek and Dixon recently measured levorphanol in plasma using electrochemical detection to a minimum level of 1 ng/mL (38). A single hexane-ethyl acetate extraction gave a recovery of 104 % with no interference seen from 6-acetylmorphine, pentazocine or heroin. The agonist/antagonist butorphanol tartrate has been assayed in urine and plasma by HPLC with UV detection at 280 nm following an ether extraction (39). A 96% recovery was determined by liquid scintillation counting of tritiated samples which were collected following HPLC.

The benzomorphan pentazocine, with agonist and antagonist activities, has been measured in blood and plasma using HPLC by Anderson et al (40). A simple dichloromethane extraction followed by a DIS-CL derivatization to overcome a low molar extinction coefficient provided a 78% effeciency. Using this technique with a levallorphan internal standard the extent of intestinal metabolism of pentazocine was determined as was the effect of enzyme inducers (41).

Meperidine pharmacokinetics have been investigated by use of a cyanopropyl column and a buffered acetonitrile-methanol mobile phase (42). The inhibitory effect of cimetidine on meperidine oxidative metabolism was demonstrated as was extraction efficiencies between 81 and 91% from serum and 77 and 93% from urine (43).

Methadone, the analgesic and abstinence syndrome suppressant, has been determined in dog plasma as a fluorescent ion-pair with dimethoxyanthranthracene-sulfonic acid (44). A cyano column was used for samples extracted with hexane followed by a post-column chloroform extraction in a two foot coil. Rat plasma and brain levels of methadone were measured by liquid scintillation counting of fractions collected following reversed-phase HPLC (45). Methanol extractions were carried out on lyophilized samples yielding results which correlated analgesia with plasma and brain levels.

The same research group has investigated the uptake of levo-alpha-acetylmethadol (LAAM) along with methadone by perfused rat lung using HPLC with UV and liquid scintillation detection (46). Ethyl acetate extractions of perfusate fractions were used to determine influx and efflux kinetics. An earlier study included the use of a normal phase system with UV detection for LAAM and metabolites in plasma and urine (47). The effect of mobile phase composition, flow rate, detection wavelength and extraction method were investigated with an optimized 95% recovery.

Propoxyphene, the phenylpropylamine of one-fifth methadone's potency, was studied in plasma and breast milk using HPLC with UV detection at 205 nm

TABLE 1A

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Opiates-Biological Studies

Column	Mobile Phase	Detection	Linearity	Reference
morphine				
20 cm silica 5µ	methanol:pH 10.2 ammonium nitrate buffer (9:1)	EC/0.6v	-	5
30 cm ODS	methanol:0.01 M KH_2PO_4 buffer (85:15)	EC/1.0v	10-200 ng/mL	6
30 cm C_{18} 10µ	70 mM NaH_2PO_4 with 1 mM Cl^- ions and 0.5 mM heptanesulfonate (pH 5.8):methanol (64:36)	EC/0.6v	25-250 ng/mL	7
30 cm C_{18} 10µ	methanol:water:ammonium hydroxide (50:50:0.1)	EC/0.65v	2-100 ng/mL	8
30 cm C_{18} 10µ	0.08M KH_2PO_4:methanol:heptane-sulfonate soln. (1000:200:2)	EC/0.64v	-	9,10
30 cm C_{18} 10µ	0.05 M sodium acetate:methanol: acetonitrile (3:1:1)	EC/0.82v and UV/254nm	-	11
ODS 5µ	acetonitrile:0.2 M sodium perchlorate in 0.005 M sodium citrate buffer pH 5.0 (100:900)	EC/0.70v	0-400 ng/mL	12
15 cm ODS 5µ	10 mM NaH_2PO_4 buffer pH 2.1 with 1 mM dodecyl sulfate:acetonitrile (74:26)	UV/210nm	20-100 ng/mL	13
30 cm C_{18} 10µ	0.07 M KH_2PO_4 with 0.5 mM EDTA: acetonitrile:methanol (87:5:8)	EC/0.79v	1-200 ng/mL	14

	25 cm	ODS	5µ	0.075 M sodium citrate/citric acid buffer pH 3.75 with 2.5% THF:methanol :acetic acid (84:10:6)	EC/0.725v	-	15
	25 cm	ODS	5µ	0.05 M citric acid:Na_2HPO_4[3:2] pH 4.2:methanol (80:20)	EC/0.80v	5-200 ng/mL	17
		ODS	5µ	0.2 M sodium perchlorate and 0.005 M sodium citrate buffer pH 5.0: acetonitrile (90:10)	EC/0.7v	1-16 ng	18
		C_{18}		sodium dodecyl sulfate	UV/210nm	-	19
	30 cm	C_{18}	10µ	acetonitrile:water:acetic acid (450:550:6) with 0.5 g sodium lauryl sulfate	fluorescence 215/340nm	-	20
		S3W silica	3µ	hexane:isopropyl alcohol: ammonia (94:4.5:0.5)	fluorescence 350/480nm	-	21
codeine							
	25 cm	ODS	5µ	0.1 M NaH_2PO_4 in acetonitrile: water (25:75)	UV/230nm	0.5-4 µg injected	22
	30 cm	silica	10µ	dichloromethane:methanol:33% ammonia (90:10:0.1)	UV/254nm	10-160 ng/mL	23
	25 cm	cyano	5µ	methanol:0.1 M phosphate buffer pH 6.8 (40:60)	UV/210nm	0.05-0.5 µg/mL	24
	25 cm	cyano	5µ	methanol:0.1 M phosphate buffer pH 6.8 (40:60)	UV/210nm	2-20 µg/mL	25
	25 cm	C_{18}		methanol:0.1 M ammonium carbonate (70:30)	UV/220nm	19-303 ng/mL	26
	10 cm	ODS	5µ	0.01 M phosphate buffer pH 3.0 with 0.1 M KBr:methanol (87.5:12.5)	EMIT and RIA	-	27
	30 cm	C_{18}	10µ	methanol:water:phosphoric acid (210:790:1.5g)	fluorescence 213/320nm	10-100 ng/mL	28
		C_{18}		methanol:water (50:50) with 0.02 M pH 5.0 ammonium acetate, 0.1 M sodium perchlorate and 10 mM sodium octyl sulfate	EC/1.15v	-	29

(continued)

TABLE 1A (continued)

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Column	Mobile Phase	Detection	Linearity	Reference
heroin				
30 cm C_{18} 10µ	methanol:0.1% ammonium carbonate-0.01 M ammonium monohydrogen phosphate buffer pH 6.98 (55:45)	UV/254nm	0.2-0.4 µg/mL	30
30 cm silica 5µ	acetonitrile:methanol:Soln A:Soln B, (75:25:0.04:0.216) where Soln A is ammonia:methanol (1:2) and Soln B is acetic acid:methanol (1:1)	UV/218nm	12.5-200 ng/mL	31
oxycodone				
RP-8 10µ	0.01 M KH_2PO_4:methanol:acetonitrile (20:30:50)	EC/1.2v	2-200 ng/mL	32
ODS	water with 1.1 g trimethyl chloride, 0.4 mL conc sulfuric acid and 0.5 mL tetramethylammonium chloride:acetonitrile (65:35)	UV/282nm	-	33
nalbuphine				
25 cm ODS 5µ	0.01 M KH_2PO_4:methanol (55:45)	EC/0.75v	-	34
15 cm C_8 5µ	0.01 M KH_2PO_4:methanol (55:45)	EC/0.75v	10-100 ng/mL	35
15 cm C_8 5µ	acetonitrile:0.348 M phosphoric acid: 1N NaOH:water (255:32:1: dilute to 1 L)	EC/0.95v	0.21-42 ng/mL	36
levorphanol				
silica	dichloromethane:methanol:ammonia (90:9.4:0.6)	UV/254nm	-	37
30 cm C_{18} 10µ	acetonitrile:0.01 M NaCl with 0.1 mM EDTA pH 4.8 (30:70)	EC/1.0v	1.25-50 ng/mL	38
butorphanol				
30 cm amino 10µ	A: 10% ethanol in benzene, B: 100% ethanol step gradient	UV/280nm and liq. scintillation	-	39

pentazocine					
30 cm C_{18}	10µ	acetonitrile:0.7% ammonium chloride pH 8 (80:20)	UV/280nm	-	40
30 cm C_{18}	10µ	acetonitrile:0.7% ammonium chloride pH 8 (80:20)	UV/275nm	-	41
meperidine					
15 cm cyano-propyl	5µ	acetonitrile:15 mM KH_2PO_4 pH 7.0: methanol (55:25:20)	UV/205nm	-	42
15 cm cyano-propyl	5µ	acetonitrile:15 mM KH_2PO_4 pH 7.0: methanol (55:25:20)	UV/205nm	10-1000 ng/mL	43
methadone					
30 cm cyano	10µ	0.025 M acetate buffer pH 3.6: acetonitrile (80:20)	fluorescence 380/445nm	-	44
30 cm C_{18}	10µ	methanol:ethanol:0.3 N diethyl-amine:water (100:100:1.85:4)	liquid scintillation	-	45
LAAM					
30 cm C_{18}	10µ	methanol:ethanol:0.3 N diethyl-amine:water (100:100:1.85:4)	UV/254 and liquid scintil-lation	-	46
30 cm silica	5µ	methanol:acetonitrile (70:30) with 0.015% ammonium hydroxide	UV/218nm	10-100 ng/mL	47
propoxyphene					
30 cm C_{18}	10µ	acetonitrile:0.002 M sulfuric acid (1:1)	UV/205nm	40-480 ng/mL	48
general					
C_{18}		methanol:0.05 M phosphate buffer pH 7 (40:60)	EC	0-300 ng/mL	49
25 cm ODS	5µ	methanol:0.05 M phosphate buffer pH 7 (40:60)	EC/dual coulometric 0.9v/0.4v	10-300 ng/mL	50

(48). The reversed-phase method employing a butyl chloride extraction, acid back-extraction and chloroform reextraction was sensitive to 20 ng/mL.

A general method for identifying and measuring opiates in biological fluids using a combination of enzyme immunoassay and LCEC has been proposed by Sunshine and coworkers (49, 50). A dual coulometric cell with an average electrolysis effeciency for four opiates of 88% was compared to a low temperature isotropic carbon working electrode. While detection limits of 5 ng/mL were found, similar to amperometric detectors, the method gave 70-94% extraction effeciencies. Figure 3 shows the chromatographic separation of seven opiates using this system with the dual coulometric detection.

Natural Products

The opiates morphine and codeine have been determined in natural products by HPLC using more straightforward techniques than in the biological studies above owing to the considerably higher drug content. Vincent and Engelke for example reported morphine and codeine content from 0.22-1.62 and 0.04-0.36 % (w/w) respectively in Papaver somniferum L. capsule using a normal phase system and UV detection at 285 nm (51). Recoveries of 99% for morphine and codeine were described following a four-fold chloroform-isopropanol extraction of basified samples which were previously treated with acetic acid. A soxhlet extraction of Papaver somniferum tissue with methylene chloride gave a total alkaloid content which was separated into phenolic and nonphenolic components (52). Four systems including isocratic adsorption, adsorption with gradient elution, reversed-phase and ion-

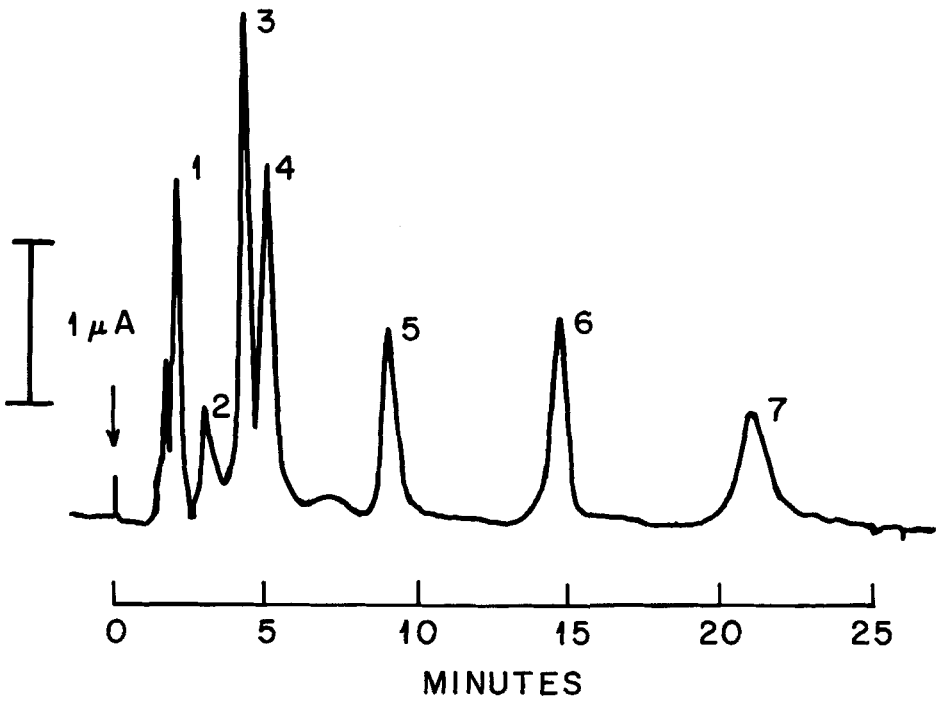


FIGURE 3. Chromatogram for LCEC determination of opiates using dual-coulometric detection cell; with w_1 set at 0.900 v and w_2 set at 0.400 v, recording the differential output (w_1 - w_2). Test mixture of 0.050 mg/L of each drug with phosphate component of mobile phase at pH 7.0. 1 = normorphine, 2 = norcodeine, 3 = morphine, 4 = hydromorphone, 5 = codeine, 6 = nalorphine IS and 7 = naloxone. Reproduced from the Journal of Analytical Toxicology by permission of Preston Publications, Inc. and with authors' permission.

pairing reversed-phase were investigated, the choice of which depended on the alkaloid content of the sample. Freeze-dried Papaver somniferum latex extracted by the method of reference 51 above was chromatographed on an NH_2 column in a weak anion-exchange mode (53). An acetonitrile:phosphate buffer mobile phase gave satisfactory separation of the six

TABLE 1B

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Opiates-Natural Products

morphine and codeine

Column		Mobile Phase	Detection	Linearity	Reference
30 cm silica	5μ	hexane:methylene chloride:ethanol: diethylamine (300:30:40:0.5)	UV/285nm	-	51
30 cm silica	10μ	A) hexane:dichloromethane:ethanol: triethylamine (300:60:60:20) B) hexane:dichloromethane:triethyl-amine (300:60:20)-gradient to ethanol	UV/280nm	-	52
30 cm C_{18}	10μ	A) water:acetonitrile:triethylamine (40:60:0.1) B) water:methanol:acetic acid (56: 44:1) with 0.005 M heptanesulfonate	UV/280nm	-	52
amino		0.025 M KH_2PO_4:acetonitrile (25:75)	UV/286nm	-	53
Nova C_{18} or radial compression C_{18}		0.01 M potassium perchlorate and 0.005 M n-butylamine at pH 3.0 with perchloric acid:acetonitrile-gradient: 10-70% acetonitrile	UV/280nm	50-700 ng	54
30 cm cyano or	10μ	1% ammonium acetate pH 5.8:aceto-nitrile:dioxane (80:10:10)	UV/254nm	-	55
30 cm C_{18}	10μ	1% ammonium acetate pH 5.8:aceto-nitrile (65:35)			
silica	5μ	1,2-dichloroethane:methanol:acetic acid:diethylamine:water (160:40: 2:1:2)	UV/254nm	-	56
30 cm silica	10μ	hexane:methylene chloride:ethanol: diethylamine (300:30:40:0.5)	UV/285nm	-	57
25 cm phenyl		acetonitrile:water:acetic acid:dimethyl-octylamine (50:950:1:0.04) gradient to: acetonitrile:water:acetic acid:dimethyl-octylamine (200:800:1:0.04)	UV/275nm	3-100% w/w	58

common alkaloids including morphine and codeine in this extract. The same latex dissolved in methanol was chromatographed on a radial compression unit with both C-18 and Nova C-18 packing which differ in the degree of end-capping (54). A six minute analysis time was proposed using a gradient from 10-70% acetonitrile at 3 mL/min.

A cyano column was shown to provide superior separation compared to a C_{18} column for alkaloids including morphine and codeine from an acid extract of gum opium (55). Average morphine and codeine content of 11.52 and 3.50 % (w/w) respectively were found with studies of buffer pH-mobile phase composition-retention times included. A similar study on opium was carried out with a simple mobile phase extraction with a silica gel column and UV detection at 254 nm (56). Alkaloids including morphine and codeine in poppy straw have also been assayed by HPLC. The effect of milling and mixing technique on morphine recovery was investigated as related to sieved particle size (57). A phenyl column with a gradient elution was used in another study of alkaloids in poppy straw (58).

Dosage Form Studies

Opiates in dosage forms have been measured using HPLC where detection limits are usually well below the assay levels as in natural products. An early report on the use of a macroporous styrene-divinylbenzene stationary phase found no column degradation when aqueous acetonitrile mobile phases were used with ammonia concentrations up to 0.05 M (59). A water extract of opium powder, USP, for morphine and codeine was used in this method without quantitation of these

drugs. Johnston et al used a reversed-phase system to determine morphine in camphorated opium tincture, BP (60). Morphine was also separated from degradation products resulting from 0.1 M HCl, 0.1 M and 0.5 M NaOH, 0.125 M NaOH with aeration and 3% hydrogen peroxide.

Analysis of morphine in morphine injection has been reported with a simultaneous assay of morphine and the preservative chlorocresol obtained using reversed-phase conditions (61). In another study common preservatives and degradation products were separated from morphine in morphine sulfate injection by an ion-pairing method with assay values for lots from six manufacturers (62). An indication of peak purity was obtained by taking spectra of collected fractions under acidic and basic conditions. The morphine degradation product pseudomorphine has been analyzed in intrathecal morphine injection where antioxidants cannot be included using a radial compression cyano column (63). Morphine sulfate injection was also analyzed by HPLC in a study on compatibility with heparin sodium (64). Incompatibility was found at morphine levels higher than 5 mg/mL which could be eliminated by dilution in 0.9% saline.

Morphine HCl and codeine HCl were determined simultaneously in tablet and injection formulations using a 5μ C_8 column (65). Precision was determined by replicate analysis of powdered tablets and in content uniformity studies for comparison to a time-consuming GLC method. Morphine sulfate and meperidine HCl 36 hr stability in parenteral nutrient formulations were measured by HPLC with water-methanol mobile phases (66). Drug degradation using 1 N NaOH, H_2SO_4

and basic ferricyanide was also carried out with separation of products from morphine and meperidine.

Codeine in dosage forms, most often in combination with other drugs, has been determined by HPLC. An assay has been developed for codeine in syrup using dioctyl sodium sulfosuccinate as pairing ion with a study of pairing ion chain length on opiate retention (67). A second study on codeine syrup used a phenyl column with a heptanesulfonate pairing ion (68). In this method codeine, guaifenesin, phenylpropanolamine and sodium benzoate were determined simultaneously using a UV detector sensitivity change midrun. Codeine has been measured in an acetaminophen-codeine product using chlorpheniramine as internal standard (69). This normal phase method was applied to tablets, capsules and elixir formulations with results reported as percent of label claim. A simultaneous assay of codeine with acetaminophen, aspirin, caffeine, phenacetin and salicylamide in tablets was carried out by reversed-phase (70). Percent recoveries from 99.1 to 100.7 were found for each component in synthetic mixtures. A similar method was developed for the simultaneous assay of codeine, acetaminophen and aspirin in tablets and suppositories (71). The tablets were extracted with methanol and filtered while the suppositories were first dissolved in hexane and then extracted with methanol.

Heroin (diacetylmorphine, diamorphine) has been measured in dosage forms usually with investigation of its stability since it gives rise to 6-acetylmorphine and morphine by successive hydrolysis steps. Poochikian and Cradock in a series of studies first investigated the hydrolysis of heroin in phosphate buffer as a function of pH using a C_{18} column (72).

The two consecutive first-order rate constants were evaluated using the stability-indicating properties of HPLC. Next heroin and cocaine were measured simultaneously in the presence of degradation products from Brompton mixtures using a pH 3.3 reversed-phase system (73). Pseudo-first-order kinetics of hydrolysis were discribed for both components with an accelerated rate of decomposition for cocaine when morphine was substituted for heroin. These studies were further extended to include buffer, salt and excipient effects on the hydrolysis kinetics (74). The stability of heroin in chloroform-water solution, a substitute for the Brompton cocktail, was also studied by HPLC (75,76). A 10 cm ODS column was used with pentanesulfonate as pairing ion to determine first-order rate constants for heroin hydrolysis.

Morphones other than morphine, codeine and heroin have also been determined in dosage forms by HPLC. Hydrocodone has been measured simultaneously with acetaminophen in tablets using an aqueous methanol mobile phase containing 0.05 N potassium nitrate at pH 4.5 (77). Chromatograms were included for hydrocodone stressed with heat, 0.1 N NaOH, 1.0 N HCl, photolysis and 3% H_2O_2 which showed separations of degradation products. Dihydrocodeine has been measured simultaneously with aspirin, caffeine and promethazine in a capsule formulation with a gradient acetonitrile-phosphate buffer system (78). A 3.37% CV was found for dihydrocodeine in eight replicate standard injections. An official method has been presented for oxymorphone in suppositories using a pH 9.1 mobile phase and SAX column (79). A chloroform extraction was used to cleanup the drug in the acid-

ified preparation containing drug and internal standard.

An HPLC assay for butorphanol tartrate injection has been described in the USP (80). A phenyl column was used with an ammonium acetate-acetonitrile-acetic acid mobile phase with a propylparaben internal standard. The benzomorphan pentazocine has been measured in an injection dosage form in a stability study using a heptanesulfonate pairing ion (81). The repackaged solutions were stable up to 360 days at room temperature. Two LC systems, one a reversed-phase ion-pairing and the second a normal phase system, were described to assay pentazocine in tablets (82). Linearity of recovery was demonstrated by peak height and area while the retention mechanism was investigated using various pairing ions. Meperidine HCl in three dosage forms including injection, tablets and syrup, has been determined by HPLC (83). The internal standards hydroxyzine or hydroxyprogesterone caproate were used to give linearity between 5 and 13 µg meperidine injected.

Methadone determination by HPLC has been described in several dosage forms. A sustained release tablet formulation was analyzed using a reversed-phase pentanesulfonate pairing ion system (84). An internal standard was used to give an average 93% recovery from actual tablet samples. Official procedures were described for methadone in oral concentrate and oral solution formulations (85,86). These used C_{18} and phenyl columns respectively with a gradient and isocratic elution. Methadone in grape Kool-Aid cocktail has been assayed by an ion-pairing method with UV detection at 230 nm (87). Linearity

TABLE 1C

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Opiates-Dosage Forms

Column		Mobile Phase	Detection	Linearity	Reference
morphine and codeine					
22 cm styrene-divinylbenzene	10µ	acetonitrile:water (48:52) with 0.019 M ammonia overall	UV/254nm	-	59
morphine					
25 cm ODS	5µ	water:acetonitrile (74:26) with 0.01 M NaH_2PO_4 and 0.001 M sodium lauryl sulfate pH 2.1	UV/280nm	15-25 µg/mL	60
10 cm RP-18	10µ	0.75% ammonium acetate pH 7.0: acetonitrile (70:30)	UV/285nm	0.2-1.4 mg/mL	61
30 cm C_{18}	10µ	methanol:0.005 M heptanesulfonate: acetic acid (280:720:10)	UV/284 and 323nm	3.2-12.2 µg/25 µL	62
radial compression cyano	10µ	0.05 M KH_2PO_4 pH 4.5:acetonitrile (80:20)	UV/240nm	5-100 µg/mL	63
15 cm ODS	5µ	0.1% NaH_2PO_4 pH 4:methanol (35:65)	UV/254nm	-	64
12 cm C_8	5µ	0.01 M phosphate buffer pH 5.0: acetonitrile (60:40)	UV/220nm	50-150% label claim	65
morphine and meperidine					
10 cm radial compress. C_{18}	10µ	morphine: 15% methanol:85% water with 0.025 M KH_2PO_4 pH 7.0 and 0.375% octyldimethylamine meperidine: 42% methanol:58% water with 0.025 M KH_2PO_4 pH 7.0 and 0.75% octyldimethylamine	UV/254nm	0.5-1.5 mg/mL 0.05-.15 mg/mL	66
codeine					
25 cm RP-8	10µ	0.005 M pairing ion and 0.01 M ammonium nitrate in acetonitrile:water (375:625) pH 3.3	UV/254nm	0.44-3.28 mg/mL	67
30 cm phenyl	10µ	methanol:water (36:62) 1% acetic acid and 0.004 M heptanesulfonate	UV/254nm	25-200% label claim	68

25 cm silica	10µ	methylene chloride:methanol:hexane:ammonium hydroxide (23.2:4.4:72.3:0.1)	UV/254nm	-	69
30 cm C_{18}	10µ	0.01 M KH_2PO_4 pH 2.3 :methanol (81:19)	UV/254nm	-	70
30 cm C_{18}	10µ	methanol:water with PIC-B-8 (1 pkg/L) (36:64)	UV/240nm	-	71
heroin					
30 cm C_{18}	10µ	0.015 M KH_2PO_4 pH 3.5:acetonitrile (7:3)	UV/235nm	-	72
30 cm C_{18}	10µ	0.015 M KH_2PO_4 pH 3.3:acetonitrile (7:3)	UV/235nm	20-200 µg/mL	73
30 cm C_{18}	10µ	0.015 M KH_2PO_4 pH 3.5:acetonitrile (7:3)	UV/235nm	10-200 µg/mL	74
10 cm ODS	5µ	0.01 M pentanesulfonate:acetonitrile: phosphoric acid (69.5:30:0.5)	UV/284nm	0.1-0.5 mg/mL	75
10 cm ODS	5µ	0.01 M pentanesulfonate:acetonitrile: phosphoric acid (69.5:30:0.5)	UV/284nm	-	76
hydrocodone					
30 cm C_{18}	10µ	0.01 M KH_2PO_4 with 0.05 N KNO_3 pH 4.5:methanol (75:25)	UV/283nm	0.035-.065 mg/mL	77
dihydrocodeine					
30 cm C_{18}	10µ	gradient- A) 0.01 M KH_2PO_4 pH 2.3, B) 60% acetonitrile in A)	UV/247nm	0.4-1.6 µg injected	78
oxymorphone					
10 cm SAX	30-50µ	0.05 M sodium borate pH 9.1	UV/254nm	-	79
butorphanol					
30 cm phenyl	10µ	0.05 M ammonium acetate:acetonitrile (3:1) pH 4.1	UV/280nm	-	80
pentazocine					
30 cm C_{18}	10µ	methanol:water pH 3.5 (66:34) with 0.005 M heptanesulfonate	UV/278nm	-	81
30 cm C_{18} or 25 cm silica	10µ 5µ	0.005 M octanesulfonate:methanol: phosphoric acid (600:400:1) chloroform:methanol:isopropylamine (960:40:2)	UV/280nm	80-120% label claim	82

(continued)

TABLE 1C (continued)

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Column		Mobile Phase	Detection	Linearity	Reference
meperidine					
30 cm C_{18}	10µ	0.02 M ammonium acetate:acetonitrile (40:60)	UV/232nm	5-13 µg injected	83
methadone					
30 cm C_{18}	10µ	pentanesulfonate:water:methanol:acetic acid (0.951 g:250:750:adjust pH to 3.5)	UV/230	5-50 µg/mL	84
25 cm C_{18}		gradient-A) formic acid:water:ammonium hydroxide (100:1898:2.5), B) acetonitrile	UV/280nm	-	85
30 cm phenyl	5-10µ	0.033 M KH_2PO_4 pH 4.0:acetonitrile (60:40)	UV/254nm	-	86
30 cm C_{18}	10µ	0.138 M pentanesulfonate pH 3.5:methanol (675:325)	UV/230nm	-	87
propoxyphene					
25 cm silica	5µ	isopropanol:hexane:water (80:20:1)	UV/220nm	0.001-1.0 mg/mL	88
30 cm ODS	10µ	KH_2PO_4:ammonium formate:water:methanol (1.361 g: 1 g:700:300) pH 6	UV/229nm	-	89

was demonstrated for a fifteen point standard curve with a CV of 1.4% for an eight-fold replicate assay.

Propoxyphene has been determined in three dosage forms including tablets, capsules and sustained release capsules using a method which permitted separation of the diastereomeric α-d- from β-dl-propoxyphene (88). Propoxyphene and acetaminophen in capsules have been s'multaneously determined by a reversed-phase method which allowed assay of the p-aminophenol degradation product of acetaminophen (89). An average recovery of 99.6% for eight samples was demonstrated by this method.

Forensic Studies

The use of HPLC in forensic chemistry has been well reviewed in the past, see references 1-3. A review of HPLC analysis of analgesics would not be complete however, without inclusion of certain recent developments in this area. J.K. Baker et al have devised a retention index system for narcotic analgesics using Hansch substituent constants (90). Two phosphate buffered mobile phases were used to separate members of the morphone, morphinan and benzomorphan classes. A methanol-aqueous ammonium nitrate mobile phase at pH 10.1 was used with silica gel pre- and analytical columns in series to determine retention characteristics of 84 drugs of forensic interest by another group (91). The separation of eleven opiates using this system is shown in Figure 4. The effect of mobile phase and pairing ion on capacity factor and selectivity for common forensic drugs was investigated by Lurie and Demchuk (92). The identification of pentazocine and tripelenamine in combination was reported by Noggle using both normal and reversed-phase methods

TABLE 1D

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Opiates-Forensic Studies

Column	Mobile Phase	Detection	Linearity	Reference
30 cm C_{18} 10µ	A) 6.6 g K_2HPO_4, 8.4 g KH_2PO_4, 1.6 L methanol and 2.4 L water pH 7.0 B) 3.3 g K_2HPO_4, 4.2 g KH_2PO_4, 2.8 L methanol and 1.2 L water	UV/ 254 and 280nm	-	90
25 cm silica S5W	methanol:aqueous ammonium nitrate buffer pH 10.1 (9:1)	UV/254nm	-	91
30 cm phenyl or 30 cm C_{18} or 30 cm cyano	methanol:water:acetic acid (40:60:1) 0.005 M alkylsulfonate overall	UV	-	92
30 cm C_{18} 10µ or	pH 3.0 phosphate buffer:methanol: acetonitrile (10:3:1)	UV/254,280 and 313nm	-	93
30 cm silica 10µ	cyclohexane:methylene chloride:methanol: diethylamine (450:40:10:0.5)			
amino-propyl	acetonitrile:0.005 M PIC A (85:15)	UV/284nm	to 4 µg injected	94
30 cm C_{18} 10µ or 25 cm ODS-3	acetonitrile:water:phosphoric acid (12:87:1) pH 2.2, 0.02 M methane-sulfonate overall	UV/254 and 220nm	62-4000 µg/mL	95
25 cm silica 7µ	hexane:dichloromethane:methanol (75:20:5) with 0.75% diethylamine	UV/227nm	-	96

25 cm silica	7µ	hexane:dichloromethane:methanol (75:20:5) with 0.75% diethylamine	UV/260nm and fluorescence 260/400nm	-	97
ODS		methanol:water:THF (60:35:5)	UV/254nm and fluorescence 280/400nm	-	98
25 cm silica	5µ	isooctane:diethyl ether:methanol: diethylamine:water (400:325:225:0.5: 15)	UV/279nm	-	99
25 cm alumina A5Y		citric acid:tetramethylammonium hydroxide:methanol:acetonitrile: water (0.01 M overall:0.01 M overall: 12.5:12.5:75)	UV	0.2-0.8 mg/mL	100
15 cm ODS or 12.5 cm C_{18} or 3 cm C_{18}	3µ	A) water: 2M NaOH:phosphoric acid: hexylamine pH 2.2 (870:30:10:7) A:methanol (60:40) or A:acetonitrile (60:40) or A:THF (60:40)	UV/210nm or fluorescence 230/340nm or EC/1.20v	-	101

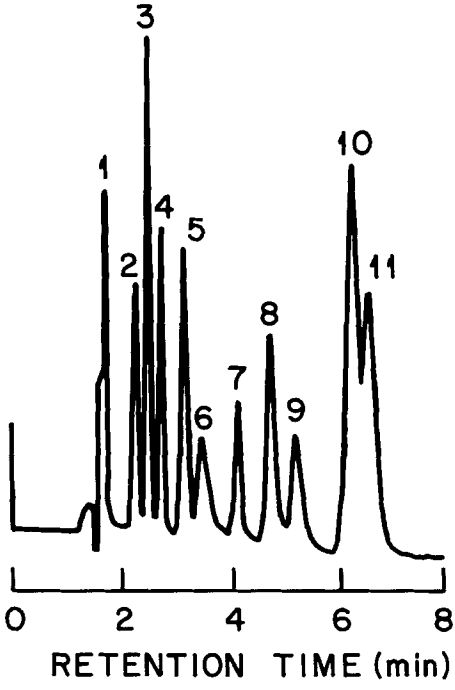


FIGURE 4. Separation of some narcotic analgesic drugs and related compounds on a silica column (Spherisorb S5W 250 x 5 mm ID). Eluent, methanol-aqueous ammonium nitrate buffer pH 10.1 (9:1, v/v). Flow rate = 2 mL/min. Detection 254 nm. Peaks: 1 = dextropropoxyphene; 2 = dipipanone; 3 = 6-monoacetylmorphine; 4 = methadone; 5 = morphine; 6 = morphine-3-glucuronide; 7 = norpethidine; 8 = dihydrocodeine; 9 = dihydromorphine; 10 = norcodeine; 11 = normorphine. Reprinted with permission Elsevier Science Publishers and the Controller of Her Britannic Majesty's Stationery Office.

(93). A detection wavelength study was included with the ratio at 254/280 nm found useful.

The use of HPLC in analyzing for active drugs, inactive excipients and processing impurities in illicit heroin preparations has been investigated. P.B. Baker and Gough used a tetrabutylammonium pairing ion with an aminopropyl column for separation of

the components of illicit heroin including codeine and morphine (94). Heroin recoveries from synthetic mixtures ranged from 97.6-101.8%. Synthetic samples and actual heroin exhibits were assayed using ODS and C_{18} columns at 220 nm (95). Common adulterants including the analgesics morphine, aminopyrine, acetaminophen, codeine, salicylamide, antipyrine, aspirin, phenacetin, meperidine and methadone were measured using absorbance ratios at 220/254 nm. Heroin samples from the Middle and Far East were investigated using a silica gel system with detection at 227 nm (96). Heroin was dissolved using tartaric acid, extracted with dichloromethane and assayed. In addition the formation of heroin from morphine plus acetic anhydride was followed by the same investigator. In another study it was possible to determine common or independent origins for heroin samples with the aid of UV and fluorescence detection for HPLC (97). Twenty-five Middle East samples gave heroin assays from 64-88%. The same detection methods were used by other workers assaying illicit heroin samples in comparison to a GC procedure (98). A simple chloroform extraction was used in this study to show the difference in heroin content of samples obtained from drug dealers and users. Two LC systems were demonstrated to measure opiate and sugar content of heroin samples with UV and refractive index detection respectively (99). The average total sugar content found from ten samples was 44.4 %. Middle East and Far East heroin samples were studied using novel slurry-packed alumina columns which separate opiates in a cation-exchange mode (100). Lurie and Allen investigated acid and neutral rearrangement compounds

resulting from the reaction of acetic anhydride with heroin congeners by means of UV, fluorescence and electrochemical detection (101). Octadecylsilane columns of 3, 12.5 and 15 cm length were utilized in comparing two analytical LC systems with the finding of highest sensitivity for fluorescence detection.

Miscellaneous Studies

Several papers have appeared in the literature concerning HPLC analysis of opiates with emphasis on detection methods. Among the methods commonly used are ultraviolet, fluorescence and electrochemical. J.K. Baker et al devised a method of relative retention times combined with UV absorbance ratios at 254/280 nm to identify most of a set of 101 drugs (102). Reversed and normal phase systems were compared in distinguishing nineteen analgesics. A rapid-scanning diode array detector was used with an aminopropyl silica column to separate heroin, 6-acetylmorphine and morphine and to investigate their zero-, first-, second- and higher order derivatives of absorbance spectra (103). As indicators of peak homogeneity, second derivatives of absorbance spectra were considered useful as were absorbance ratios which could be continuously monitored and plotted. Three-dimensional pseudo-isometric plots of absorbance, wavelength and time, as shown in Figure 5 for heroin, 6-acetylmorphine, morphine and p-hydroxybenzoic acid, were informative but took 30-45 min to complete.

A post-column fluorescence derivatization and detection scheme was investigated for morphine using ODS columns and a basic potassium ferricyanide reagent (104). While an 80% decrease in response resulted from quenching and incomplete derivatization, a

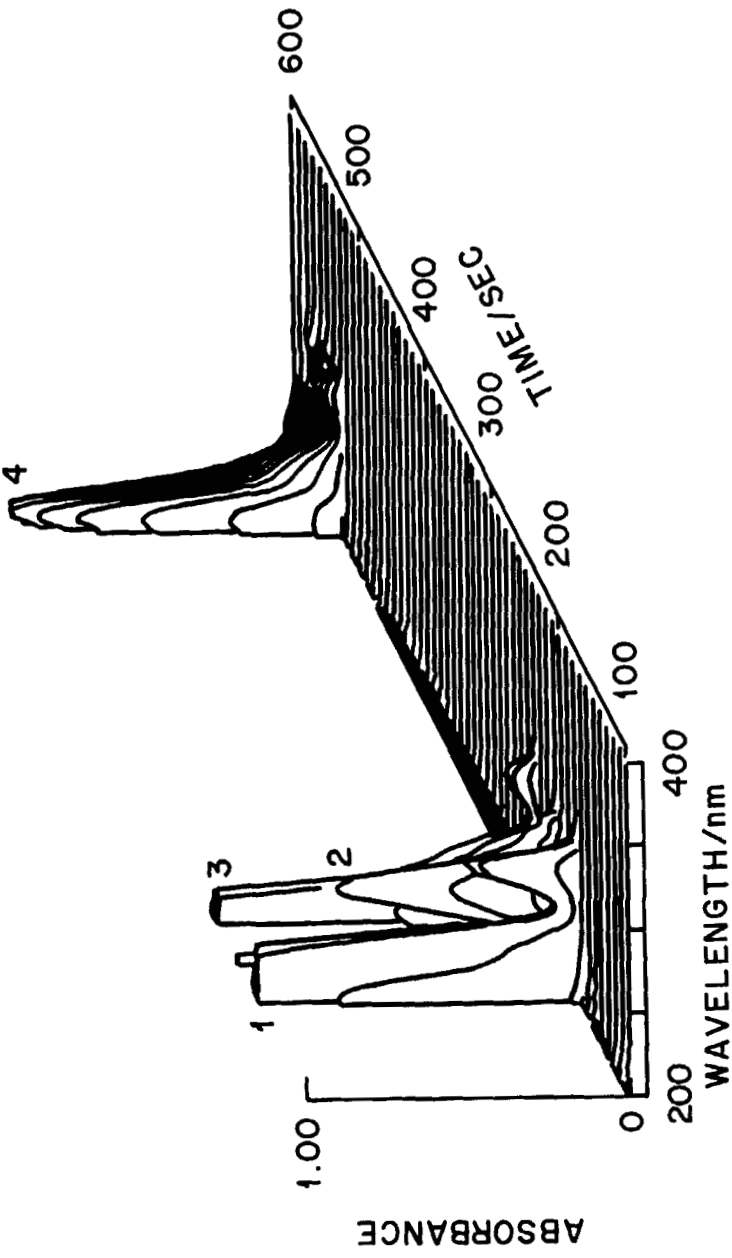


FIGURE 5. Pseudo-isometric (A,λ,t) presentation of sequential spectra captured at 2.5 sec intervals during elution. Hidden lines and superfluous baseline spectra have been omitted for clarity. Injection volume, 10 µL. (1) Diacetylmorphine (5.0 µg); (2) p-hydroxybenzoic acid (internal standard, 0.50 µg); (3) 6-acetylmorphine (2.0 µg); and (4) morphine (4.0 µg). Reprinted with permission Elsevier Science Publishers and authors.

detection limit of 10 ng morphine was found. Nelson extended this work to study the effect of micelle formation on morphine fluorescence derivatization (105). Linearity of detection was demonstrated for morphine and dihydromorphine from 100 ng to 2 µg injected with the nonionic surfactant Triton X-100 giving the largest increase in sensitivity.

Electrochemical detection for opiates has been investigated in areas other than biological measurement. The analgesics morphine and oxymorphone were detected amperometrically at 0.8 v in a reversed-phase system by one group while codeine and pentazocine were not detected (106). A study of opiate structure-amperometric reactivity revealed the importance or lack of importance of the 3-hydroxyl group, the 4-5 furan link, the 14-hydroxyl and the C ring of morphinans (107). Analgesics investigated in this and a naloxone dosage form study on ODS columns included hydromorphone, levorphanol, cyclazocine and oxycodone (108).

In a comprehensive study on the normal phase chromatography of 462 compounds, Jane et al compared ultraviolet, fluorescence and electrochemical detection (109). Included among this series were the analgesics: codeine, cyclazocine, etorphine, fentanyl, heroin, hydrocodone, hydromorphone, levorphanol, methadone, morphine, oxycodone, oxymorphone, pentazocine, meperidine and phenazocine where markedly tailing peaks for most of the morphones in this group were found. Methanolic perchloric acid or methanolic ammonium perchlorate solutions were the most useful eluents with the silica gel columns studied.

Other studies on HPLC analysis of opiates could not be conveniently classified. Hansen, for example, investigated the use of an aqueous phosphate buffered-methanol mixed mobile phase with a silica gel column dynamically coated with cetyltrimethylammonium ions (110). Morphine was retained in this system by a mixed mechanism of reversed-phase partition and cation-exchange with the silica gel surface. He further studied the retention of morphine and codeine in a silica gel partition system using mixed aqueous mobile phases (111). The effect of mobile phase pH, organic component, modifying amine and added acid were studied. A statistical factorial design approach was used to optimize morphine and codeine retention in a reversed-phase ion-pairing system (112). Variables included the methanol-water ratio, pH, buffer concentration and pairing ion concentration.

An investigation of the synthesis of 10-oxo-morphine, a morphine degradation product, utilized HPLC to separate these compounds and the partially oxidized 10-hydroxy derivative (113). Retention orders were compared with TLC R_f values and the merits of UV vs. fluorescence detection were discussed. The reversed-phase separation of 14 alkaloids including codeine was studied in view of their retention mechanism (114). The k' values were found to be linearly related to the log of the buffer concentration.

Lurie et al studied the reversed-phase separation of 26 fentanyl analogs and homologs with overlapping resolution mapping (115). The analogs were readily distinguished in 20 minutes run time using two UV detectors in series set at 215 and 230 nm. A further study of these analogs revealed that selectivity

TABLE 1E

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Opiates-Miscellaneous Studies

Column	Mobile Phase	Detection	Linearity	Reference
30 cm C_{18} 10µ or 30 cm silica 10µ	0.025 M NaH_2PO_4:methanol (2:3) pH 7.0 methanol:2 N ammonia:1 N ammonium nitrate (27:2:1)	UV/254 and 280nm	-	102
aminopropyl silica	acetonitrile:0.005 M tetrabutylammonium phosphate (85:15)	UV/diode array	0-1 mg/mL	103
10 cm ODS 10µ or 10 cm ODS 8µ or 20 cm Kieselgel 5µ	methanol:0.1 M KBr (12.5:87.5) pH 3 as above methanol: 2 M ammonium hydroxide: 1 M ammonium nitrate (30:20:10)	UV/254nm or fluorescence 323/432nm	-	104
10 cm ODS 8µ	methanol:0.1 M KBr (12.5:87.5) pH 3, with Triton X-100 4% w/v	fluorescence 324/430nm	100ng-2 µg injected	105
30 cm C_{18} 10µ	methanol:water with 50 nM tetrabutylammonium hydroxide pH 6.1 (20:80)	UV/254nm and EC/ .8 v	5-100 ng injected	106
25 cm ODS-3 10µ	water:methanol:acetonitrile:phosphoric acid: 0.1 M Na_2EDTA (600:200:200:1:1), 0.0028 M octanesulfonate overall	EC/.95v	-	107
25 cm ODS-3 10µ	water:methanol (550:450) with 0.01M KH_2PO_4 and 0.0028 M octanesulfonate overall pH 5.9 or water:methanol:acetonitrile:phosphoric acid (600:200:200:1) with 0.0028 M octanesulfonate and 0.0001 M Na_2EDTA, pH 2.4 overall	EC/.85v	0.1-1000 ng injected	108

25 cm ODS-3	10μ	water:methanol:phosphoric acid (550:450:1) 0.017 M sodium chloride and 0.0031 M octanesulfonate overall pH 2.7	UV/ 229nm	0.2-2000 ng injected	108 cont'd
12.5 cm silica or S5W 25 cm		ammonium perchlorate (20 mM) 60 mL methanolic NaOH (0.1 M) pH 8.3 or methanol: 0.02% perchloric acid (60:40)	UV/205nm or fluorescence 250/470nm or EC/ 0.9-1.2v	-	109
15 cm silica 60	5μ	methanol:0.2 M potassium phosphate pH 7.5:water(50:5:45) with 0.0025 M cetyltrimethylammonium bromide	UV/254nm	-	110
15 cm silica	5μ	acetonitrile:water:acetic acid: diethylamine (10:90:0.5:0.5)	UV/254nm	-	111
30 cm C_{18}	10μ	methanol:water:camphorsulfonic acid 0.005 M:phosphate buffer 0.05 M (35:65) with variation	UV/254nm	-	112
25 cm RP-18	7μ	methanolic 0.05 M ammonium hydroxide: water (65:45) or (35:65)	UV/370 and 285 nm	-	113
15 cm C-18 or 25 cm C-18	5μ 7μ	methanol: NaH_2PO_4 buffer 0.025 M (90:10) to (70:30) pH 3-8	UV	-	114
25 cm ODS-3	10μ	phosphate buffer [water:2 N NaOH: phosphoric acid][16:3:1]:acetonitrile or :methanol or :THF	UV/230 and 215nm	-	115
25 cm ODS-3 or 15 cm polystyrene-divinylbenzene	10μ 10μ	as above	UV/254nm	-	116

on a silica based ODS column was nearly identical with that of a polymer based column (116). Equations were developed which related group contributions to connectivity indexes and mobile phase composition.

II. ANTI-INFLAMMATORY ANALGESICS

A. Salicylate Derivatives

Salicylates were among the first anti-inflammatory analgesics which now could be classified as nonsteroidal anti-inflammatory drugs (NSAID). They consist of aspirin (acetylsalicylic acid), salicylamide and salicylic acid in various salt forms. They have been chromatographed by HPLC alone and in combination with other drugs for the purpose of measuring their levels in biological fluids, in conjunction with pharmacological or biopharmaceutical studies, or in dosage forms and bulk drugs.

Biological Studies

Salicylic acid as an active analgesic and metabolite of aspirin has been measured alone by HPLC in plasma and serum in several studies. A nonextraction method gave satisfactory linearity for salicylate with coefficients of variation from 0.8-2.5% using 3 µL injected from capillary pediatric blood samples (117). The method compared well with a fluorometric procedure in assaying spiked plasma samples. Another direct injection method gave a detection limit of 40 ppb in serum using a long-chain aliphatic amine column (118). Serum deproteinization with 0.3 M perchloric acid did not affect recovery of salicylic acid adversely. Serum salicylate has been measured in a study by T. Higuchi *et al* using a column-switching approach which allowed for simultaneous assays of arabinofuranosyl-

cytosine, arabinofuranosyluracil and salicylate (119). The rapid reversed-phase method gave a 113% salicylate recovery at 40 µg/mL. An ether extraction of plasma was used to prepare samples for HPLC assay in a pharmacokinetic study involving different dosage forms of salicylic acid (120). A 0.05 mmole/L detection limit was found for the method which was also used for a dissolution study on film coated and controlled-release tablets.

Several studies have simultaneously measured salicylic acid and its metabolites, salicyluric and gentisic acids in biological samples. Rowland and coworkers used a dual pump nongradient reversed-phase system to analyse plasma samples for these three constituents (121). Recoveries ranged from 98-104% at 200-300, 3-15 and 8-24 µg/mL for salicylic, gentisic and salicyluric acids respectively. Urine content of these compounds was also studied by the same group with an isocratic system which gave 92-113% recoveries (122). An acetonitrile precipitation of proteins was also used here before direct sample injection. Levels of these compounds in rodent blood, urine, feces and embryo tissue were assayed by a method involving mobile phase extractions (123). Recoveries from 91-101% for salicylic acid were demonstrated with a working range of 0.5-2.5 µg injected for each component. A pharmacokinetic study of salicylic acid and metabolites in urine and plasma compared conventional aspirin, enteric coated aspirin and enteric coated aspirin granules by HPLC (124). Minimum detectable limits for salicylic acid in plasma and urine were 30 and 10 µmole/L respectively. A recent study compared salicylic acid determination by a reversed-

phase LC system with the Natelson colorimetric method in patients with Reye's syndrome (125). No false elevation of results by the colorimetric method were found based on the HPLC data between 2 and 16 µg/mL.

The simultaneous HPLC assay of aspirin and metabolites including salicylic, salicyluric and gentisic acids in biological fluids has been reported. Problems encountered in carrying out these procedures involve inhibition of plasma and erythrocyte esterase activity, avoidance of salicylic acid evaporation in extraction steps and choice of protein precipitation or extraction methods. A limit of detection of 0.5 µg/mL for aspirin, salicylic and salicyluric acids in plasma was found in a method which avoided salicylic acid sublimation (126). A similar reversed-phase method with a methylene chloride extraction for plasma gave 1-6% CV values for aspirin standards added to plasma (127). Acetonitrile deproteination and physostigmine enzyme inhibition were used for plasma samples making direct injection of supernatant possible in another study (128). Lo and Bye used a fluoride enzyme inhibition along with a chloroform extraction at pH 1 with evaporation in an ice bath to prevent salicylic acid sublimation (129). A 40^{o} column temperature gave chromatography with a 6.4% CV for aspirin at 1 µg/mL in plasma. A rapid method for aspirin and metabolites in plasma involved a protein precipitation with 30% perchloric acid and direct injection into the reversed-phase system (130). Urine values were also obtained following a 3 hour HCl hydrolysis step. An increased sensitivity of 10-fold over previous reports was claimed for plasma aspirin in a method using fluoride enzyme inhibition

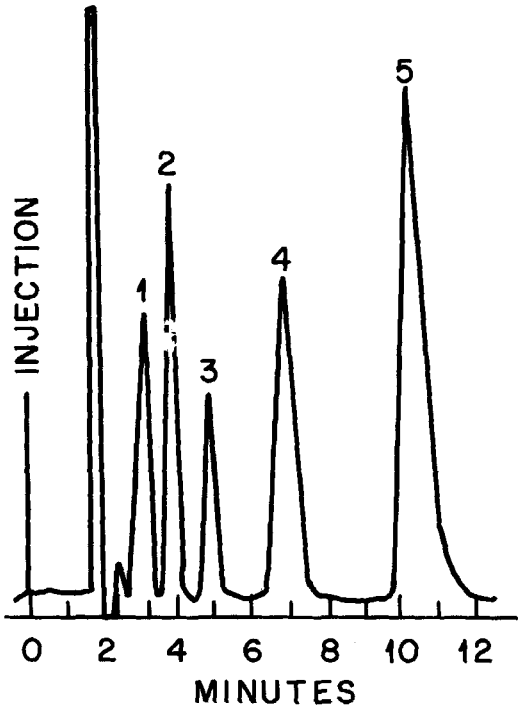


FIGURE 6. Chromatogram of salicylic acid in blood. Key: (1) gentisic acid; (2) salicyluric acid; (3) aspirin; (4) salicylic acid; (5) o-toluic acid. The concentrations for all components are 5 µg/mL in blood. Reproduced with permission of the copyright owner, the American Pharmaceutical Association.

and ether/hexane extraction (131). Another study utilized 238 and 305 nm to monitor plasma and urine aspirin and metabolite levels to give a 0.5 µg/mL limit of detection (132). Figure 6 shows the separation of salicylate metabolites obtained here from a blood sample. Average percent recoveries for aspirin, salicyluric, salicylic and gentisic acids were 99, 94, 91 and 62% respectively. An ethyl acetate extraction of fluoride stabilized plasma

samples was used with evaporation in an ice bath to give similar detection limits and recoveries as above by another worker (133). Aspirin stability in whole blood and plasma was studied at 0^{o} and 37^{o} using a reversed-phase procedure with detection at 237 nm (134). The influence of the extraction procedure, mobile phase, sample solvent and trace ions on peak shape were also investigated. A rapid method for analysis of aspirin, salicylic, salicyluric and gentisic acids in plasma and urine was developed using a C_8 column (135). Protein was precipitated with 30% perchloric acid to yield recoveries between 80 and 86% for aspirin and salicylic acid. A dichloromethane extraction of acidified plasma samples gave aspirin and metabolite levels measured at 237 nm by Mays et al (136). Recoveries of aspirin were 92% from plasma and 98% from urine with 0.2 µg/mL detection limits in each case. Another high sensitivity aspirin pharmacokinetics study (detection limit of 0.01 µg/mL) used a C-18 column at 47^{o} (137). A dual channel recorder with one input at 10 mv and the second at 100-500 mv depending on drug concentration was utilized in order to determine low levels of aspirin in the presence of high levels of salicylic acid.

Analysis of salicylates in combination with other analgesics in biological fluids has also been investigated by HPLC. A rapid chloroform/isopropanol extraction of plasma followed by evaporation, dissolution in methanol and injection directly into a reversed-phase system required a column temperature of 40^{o} in one study (138). In another an ion-pairing system was used with flow programming to measure

TABLE 2A

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Salicylate Derivatives-Biological Studies

Column	Mobile Phase	Detection	Linearity	Reference
salicylic acid				
30 cm C_{18} 10µ	methanol:0.02 M nitric acid (1:1)	fluorescence 300nm excit.	6.25-500 µg/mL	117
15 cm aliphatic amine 4-6µ	0.025 M perchloric acid :water: trioctylamine	UV/235nm	10-20 ng injected	118
10 cm RP-18 5µ	0.0068 M ammonium hydroxide: 0.01 M formic acid:20% acetonitrile	UV/254	1.25-80 µg/mL	119
30 cm C_{18} 10µ	methanol:0.1 M potassium phosphate buffer pH 3.5 (40:60)	UV/280nm	-	120
30 cm C_{18} 10µ	A) acetic acid:water (5:95), B) methanol,A:B (82:18)	UV/313nm	0.2-2000 µg/mL	121
25 cm ODS 5µ	butanol:sodium sulfate:acetic acid: water (2:10 g:5:83)	UV/313nm	2-163 µg/mL	122
30 cm C_{18} 10µ	methanol:1% acetic acid (40:60)	UV/296nm	0.0125-.0625 µg/mL	123
10 cm RP-8 10µ	acetic acid:acetonitrile:water (4:12: 84)	UV/314nm and fluorescence 315/420nm	-	124
30 cm C_{18} 10µ	acetic acid:methanol:water (41:180: 779)	UV/313nm	-	125
salicylic and acetylsalicylic acids				
30 cm C_{18} 10µ	acetonitrile:0.05% phosphoric acid (30:70) pH 2.5	UV/237nm	0.5-300 µg/mL	126
30 cm C_{18} 10µ	methanol:1% acetic acid (60:40)	UV/300nm	5-50 µg/mL	127

(continued)

TABLE 2A (continued)

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Column		Mobile Phase	Detection	Linearity	Reference
30 cm C_{18}	10μ	acetic acid:methanol:water (5:22:73)	UV/280nm	2-30 μg/mL	128
25 cm RP-18	10μ	methanol:0.072% phosphoric acid (55:45)	UV/234nm	0.5-20 μg/mL	129
30 cm C_{18}	10μ	acetonitrile:0.03% phosphoric acid (30:70) pH 2.5	UV/237nm	0.5-200 μg/mL	130
25 cm ODS	5μ	water:phosphate buffer pH 2.5, 0.2 M: acetonitrile (35:40:25)	UV/234 and 303nm	0.05-1 μg/mL	131
30 cm C_{18}	10μ	water:methanol:acetic acid (64:35:1)	UV/238 and 305nm	0.5-200 μg/mL	132
15 cm RP-18	5μ	methanol:water pH 3.0 (40:60)	UV/280nm	0.5-500 μg/mL	133
25 cm ODS	5μ	acetonitrile:5% acetic acid (20:80)	UV/237nm	1-5 μg/mL	134
25 cm C_8	10μ	methanol:0.1% KH_2PO_4 pH 3.9 (35:65)	UV/235 and 313nm	1-500 μg/mL	135
25 cm C_{18}	5μ	pH 2.5 0.05 M phosphate buffer: methanol:acetonitrile (68:16:16)	UV/237nm	0.2-100 μg/mL	136
30 cm C_{18}	10μ	phosphoric acid:1-butanol:methanol: water (0.13:10:270:720)	UV/234nm	0.025-5 μg/mL	137

salicylate analgesic mixtures					
30 cm ODS or 30 cm C_{18}	10µ	2-propanol:0.02 M phosphoric acid pH 2.9 (30:970)	UV/248nm	50-1000 µg/mL	138
30 cm C_{18}	10µ	methanol:water:PIC-A (30:70:1 pkg)	UV/254nm and fluorescence 305/320nm	1-100 µg/mL	139
30 cm C_{18}	10µ	0.1 M KH_2PO_4 pH 4.0 :acetonitrile (902.5:97.5)	UV/254nm	to 1 µg/mL	140
30 cm C_{18}	10µ	methanol:5% acetic acid (60:40)	UV/248nm	25-700 µg/mL	141
25 cm C_{18}	5µ	0.05 M ammonium monochloroacetic acid pH 3.2 and 0.1 M sodium perchlorate in water:methanol (1:1) or step gradient-40 to 60% methanol	EC/0.75 and 1.15v	2-50 µg/mL	142
15 cm cyano or 15 cm C-18 or 25 cm C-18	5µ 5µ 10µ	0.05 to 0.08 M sodium dodecylsulfate pH 3.0 with phosphate buffer	UV/254nm and fluorescence 336/370nm	50-150 µg/mL	143
salicylamide					
30 cm C_{18}	10µ	acetic acid:methanol:water (1:15:84) or 0.003 M tetrabutylammonium hydroxide in methanol:7% acetic acid (8:92)	UV/254 or 313nm	-	144
15 cm phenyl and 12 cm C_{18}	5µ 5µ	0.01 M phosphate buffer pH 2.2	UV/296nm	0.07-.3 µg/mL	145
		step gradient: A) 12 mM tetrapentyl ammonium chloride and 10 mM pH 6.2 phosphate buffer:methanol (60:40), B)= A) with 50% methanol	UV/230nm		

salicylic acid and acetaminophen simultaneously in 100 µL plasma samples from febrile children (139). Ultraviolet detection at 254 nm for APAP in series with fluorescence detection for salicylic acid made pharmacokinetics measurements possible for each. An acetonitrile protein precipitation for aspirin and salicylic acid analysis and an ethyl acetate extraction for acetaminophen were used on plasma samples in another report (140). Using the same precipitation method acetaminophen and salicylic acid were assayed simultaneously by another group (141). The application of electrochemical detection to the assay of salicylate, acetaminophen, phenacetin, salicylamide, methyl salicylate, naproxen and codeine was studied by Kissinger and coworkers with parallel-dual glassy carbon electrodes (142). Serum and urine samples were extracted as above with dilution required for the urine to give recoveries between 97 and 102% for salicylate. The advantages of micellar chromatography using solutions of SDS as the mobile phase with C-18 or cyano columns was demonstrated for therapeutic drug monitoring by direct serum injection (143). Aspirin and acetaminophen among others were measured in this way with linearity from 50-150 and from 5-30 µg/mL found respectively.

Salicylamide, the amide derivative of salicylic acid, is an active analgesic and antipyretic agent. As such, measurement of its levels in biological fluids along with those of its metabolites would be of interest in relation to biopharmaceutical parameters. Assays of salicylamide and its glucuronide and sulfate metabolites as well as gentisamide and its conjugates in plasma and urine were performed using two separate

mobile phases with a C_{18} column (144). Low concentrations of salicylamide in serum or saliva required an ether extraction and detection at 313 nm. The same wavelength was used for gentisamide and its conjugates with recoveries up to 95% found. An increased sensitivity for blood and urine salicylamide levels was reported using an ethyl acetate extraction procedure with a 15 cm phenyl column (145). A step-gradient was utilized with a solvent switching valve and UV detection at 230 nm for conjugated metabolites. Salicylamide plasma levels to 75 ng/mL were assayed by this method with species differences demonstrated in metabolite profiles.

Dosage Form and Bulk Drug Studies

The analysis of aspirin, its degradation products and related analgesics in dosage forms has most frequently been accomplished using reversed-phase techniques as was done for the biological studies above. In the application of mixed aqueous mobile phases however, a major problem to be dealt with is the instability of aspirin, which can readily undergo hydrolysis in sample preparation solvents as well. The aspirin content of aspirin powder, tablets and suppositories was measured using ethanol as diluent with injection into a C-18 system immediately after preparation (146). A pH 2.3 mobile phase was included to give increased aspirin stability. Kirchhoefer also assayed aspirin and salicylic acid in tablets immediately after sample preparation which involved addition of methanol, formic acid and internal standard solution in mobile phase (147). Series UV absorbance and fluorescence detectors were used to assay aspirin and salicylic acid respectively

with results compared to the current USP procedures. Aspirin in tablets was assayed in another study with a silica gel column and heptane-acetic acid mobile phase which gave 0.02% aspirin hydrolysis/hour after final dilution with chloroform (148). The process impurities salicylic, salicylsalicylic and acetylsalicylsalicylic acids were detected at 300 nm for increased sensitivity. Another normal phase system was proposed for assaying aspirin tablets using chloroform saturated with citric acid plus formic acid as sample preparation solvent (149). A hydrolysis rate of 0.002%/hr was found with linearity from 70-130% of the label amount of aspirin and 0.1-5.0% for salicylic acid. Buffered tablets were also assayed by this procedure with a prior adsorption step on a siliceous earth column. A reversed-phase study on aspirin and salicylic acid in tablets compared sample solvents to obtain maximum stability (150). The combination oxalic acid-acetic acid and acetic anhydride gave optimal performance with observations on peak shape related to sample solvent and component resolution related to mobile phase pH. The sample solvent acetonitrile:methanol:phosphoric acid (92:8:0.5) was found by another group to stabilize aspirin tablets prior to reversed-phase chromatography (151). This gave 0.01%/hr decomposition of aspirin with linearity between 75 and 127% of the label claim for aspirin and 0.8-6.2% for salicylic acid. An official method for aspirin in tablets uses an ion-pairing mobile phase with chloroform:formic acid:acetonitrile (99:2:99) as sample solvent to overcome the same problem (152).

Additional studies on aspirin analysis from dosage forms have been carried out by HPLC. An early

report gave a method for the simultaneous determination of acetylsalicylic anhydride, acetylsalicylsalicylic acid, salicylic acid and aspirin in tablets using a reversed-phase system with detection at 240 nm (153). Kirchhoefer and coworkers in a series of papers discussed methods to determine acetylsalicylsalicylic acid, salicylsalicylic acid, salicylic acid and aspirin in aspirin products by reversed-phase. The initial procedure isolated acetylsalicylic anhydride in a pH 11.3 benzene extract with the other components in the aqueous phase. This was then acidified and reextracted with benzene (154). The anhydride was determined by the colorimetric α-benzamidocinnamate-pyridine test while the others were determined chromatographically at 254 nm. The second report outlined a reversed-phase method for salicylic acid determination in aspirin tablets and bulk powder using an external standard (155). Results were compared to the USP method and a colorimetric procedure for tablets from 16 manufacturers. Finally 172 tablet formulations were examined using the chromatographic conditions of the previous paper for assaying acetylsalicylsalicylic acid and salicylsalicylic acid (156). Acetylsalicylic anhydride again had to be determined by the colorimetric procedure because of its instability in mobile phase. Pankey and Pfeiffer on the other hand developed a rapid normal phase method to determine salicylic acid, salicylsalicylic acid, acetylsalicylic anhydride and acetylsalicylsalicylic acid in aspirin simulatneously (157). Aspirin spiked with the four process impurities is shown in Figure 7 where it was necessary to inject samples immediately after preparation. Tablet assays were also performed

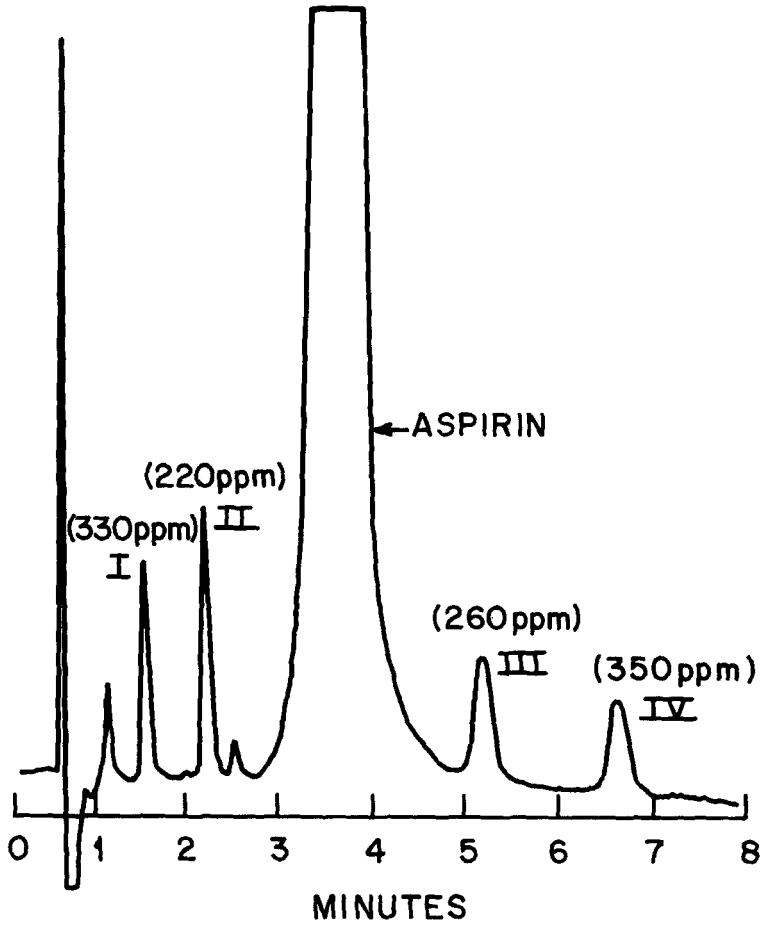


FIGURE 7. Analysis of spiked aspirin. Column, 4.6 x 150 mm Zorbax-SIL; flow rate, 3.0 mL/min; injection, 10 µL; detector, 0.016 aufs at 254 nm; mobile phase, hexane-chloroform-acetic acid (80:19:3). Key: (I) salicylic acid; (II) salicylsalicylic acid; (III) acetylsalicylic anhydride and (IV) acetylsalicylsalicylic acid. Reprinted with permission of the copyright owner, the American Pharmaceutical Association.

using the silica gel column which had been activated with a 2,2-dimethoxypropane solution.

The chromatographic separation of components in combination analgesic products has been studied by means of diverse types of column packing material. The use of polymer resins as packing for separating analgesics and tranquilizer compounds was studied including styrene-divinylbenzene, methyl methacrylate polymers and copolymers with and without hydroxymethyl substitutions (158). Mobile phase flow rate and pH were varied to give aspirin k' values from 0.05 to 2.98. Normal phase methods were developed for analgesic-muscle relaxant formulas including aspirin-meprobamate using cyano and silica gel columns (159). Other analgesics included in this study were acetaminophen and phenacetin. Two groups reported on the reversed-phase ion-pairing separation of common analgesic mixtures including aspirin (160,161). Both used tetrabutylammonium phosphate in systems which also separated acetaminophen, phenacetin, salicylamide and salicylic acid. The effect of sample solvent on analgesic peak shape as well as mobile phase composition on retention were studied using an ODS column (162). It was determined that the use of mobile phase as sample solvent did not necessarily maximize column efficiency. The advantages of high speed chromatography were investigated on an aspirin, salicylic acid, caffeine, propyphenazone mixture (163). A 5 μ RP-18 column of 2.1 mm (i.d.) was shown to give enhanced sensitivity over a 4.6 mm (i.d.) column completing a separation in 60 sec at a flow rate of 1.7 rather than 8.0 mL/min.

TABLE 2B

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Salicylate Derivatives-Dosage Form and Bulk Drug Studies

Column	Mobile Phase	Detection	Linearity	Reference
aspirin				
30 cm C_{18} 10µ	0.01 M KH_2PO_4 pH 2.3:methanol (80:20)	UV/300nm	-	146
25 cm C_{18} 10µ	acetic acid:water:methanol (15:460:525)	UV/254nm and fluorescence 325/425nm	-	147
25 cm silica 10µ	heptane:acetic acid (95:5)	UV/300nm	0.065-.65 mg/mL	148
25 cm silica 5µ	chloroform:methylene chloride:acetonitrile:formic acid (700:300:30:4)	UV/300nm	7.8-78 µg/mL	149
25 cm ODS 10µ	$NaH_2PO_4 2H_2O$:water:methanol:acetonitrile (31.2 g:1400:600:10) pH 3.75	UV/299nm	-	150
15 cm C_{18}	water:acetonitrile:phosphoric acid (76:24:0.5)	UV/295nm	75-127% label claim	151
30 cm C_{18}	heptanesulfonate:water:acetonitrile (2 g:850:150) pH 3.4	UV/280nm	-	152
25 cm C_{18} 10µ	water:methanol (45:55) 1% acetic acid	UV/240nm	-	153
or				
50 cm silica 37µ	light petroleum:ethyl acetate:acetic acid (85:14:1)	UV/306nm		
25 cm RP-18 10µ	acetic acid:methanol:water (12.5:450:538)	UV/254nm	-	154
or				
30 cm C_{18} 10µ				
25 cm C_{18} 10µ	methanol:water:acetic acid (54:46:2.5)	fluorescence 325/425nm	-	155
25 cm C_{18} 10µ	methanol:water:acetic acid (54:46:2.5)	UV/254nm	-	156
15 cm silica 6µ	hexane:chloroform:acetic acid (80:19:3)	UV/254nm	0.2-10 mg/mL	157

aspirin analgesic mixtures

styrene-divinyl benzene 10µ hydroxymethyl SDVB 10µ methylmethacryl-ate 20µ or RP-18	methanol:acetonitrile:ammonia solution mixtures	UV/254nm	-	158
30 cm silica 10µ or 30 cm cyano 10µ	chloroform with carbon tetrachloride, hexane, butyl ether or methylene chloride; carbon tetrachloride with methylene chloride or THF with toluene, hexane or butyl ether	RI	-	159
30 cm C_{18} 10µ	methanol:water (40:60) pH 6.8 with 0.01 M tetrabutylammonium	RI and UV/254nm	0.13-.52 mg/mL	160
25 cm C_{18}	methanol:0.025 M pH 7 phosphate buffer (45:55) and gradients	UV/254nm	-	161
25 cm ODS-2 10µ	acetonitrile:acetic acid:water (25:5:70)	UV/275nm	-	162
10 cm RP-8 5µ or 3 cm RP-18 5µ	methanol:water:phosphoric acid (35:70:3)	UV/295nm	-	163
30 cm C_{18} 10µ	A) 0.01M KH_2PO_4 pH 2.3:methanol (81:19) B) 0.01 M KH_2PO_4 pH 4.8: methanol (81:19)	UV/254 and 285nm	-	164
25 cm C_8 or 30 cm C_{18} 10µ or 25 cm C_{18} 5µ	methanol:buffer (1% acetic acid) (40:60)	UV/254nm	-	165
30 cm C_{18} 10µ	methanol:water:acetic acid (35:65:4)	UV/254nm	-	166
30 cm C_{18} 10µ	methanol:0.75% acetic acid (1:3)	UV/254 and 280nm		167

Several commercial combination analgesic tablet assay procedures were developed employing reversed-phase columns. Gupta separated aspirin, acetaminophen, caffeine, codeine phosphate, phenacetin and salicylamide showing the effect of mobile phase pH on retention characteristics (164). A synthetic mixture gave 100.5% average recovery for four components using water as sample solvent. Two methods have been described for HPLC separation of analgesic mixtures in over-the-counter products as experiments in undergraduate labs. In one study three columns were investigated for this separation including Zorbax C_8, μBondapak C_{18} and Ultrasphere-octyl (165). The effect of mobile phase composition was illustrated on column performance characteristics including the number of theoretical plates, k' and α values and resolution factors. The second study used a methanol-water-acetic acid mobile phase for the separation of aspirin, acetaminophen, salicylamide, phenacetin and caffeine (166). Another report described the separation of these compounds in a mixture also including chlorpheniramine, phenylephrine and salicylic acid (167). In it the sample was dissolved in mobile phase, filtered and chromatographed within one hour to minimize aspirin hydrolysis.

B. p-Aminophenol Derivatives

The second major class of anti-inflammatory analgesics is the p-aminophenol derivatives including acetaminophen (APAP, paracetamol) and its analog phenacetin. Quantitation of these drugs and their metabolites has frequently been performed by HPLC. Biological studies including pharmacckinetics in man

and animals far outweigh dosage form studies although several of the latter have been reported.

Biological Studies

Acetaminophen levels in human serum, plasma, blood and tissue have been measured by normal and reversed-phase methods with ultraviolet and electrochemical detection. Macro- and micromethods were presented for APAP quantitation depending on available sample volume using a spherical silica gel column and UV detection (168). A reversed-phase UV detection method employing an ethyl acetate extraction following a protein precipitation measured APAP from 1-180 µg/mL in serum (169). Coefficients of variation from 8-10% were found in day-to-day precision studies. A method to measure APAP in blood and postmortem tissue was described in which 95% ethanol was added to samples prior to the ethyl acetate extraction (170). Recoveries from blood were 99 and 106% depending on whether an internal or external standard was used. Another reversed-phase UV detection method for APAP in serum was proposed by Snyder and associates (171). A FAST-LC system was used which permitted the processing and analysis of 15 samples per hour. With an organic extraction solvent of 25% isopropanol/chloroform, recoveries from 96-100% were found at 43-150 µg/mL.

Electrochemical detection has also been utilized to assay human serum, plasma and CSF for APAP. A methylene chloride-isopropanol-ether extraction of serum gave 80-90% recovery with linearity between 20 ng/mL and 20 µg/mL (172). APAP in CSF has been separated from HVA and 5-HIAA by HPLC and found to vary from 9-150 ng/mL (173). An ethyl acetate

extraction of plasma for APAP was presented with electrochemical detection which resulted in an 87% recovery and a minimum detectable concentration of 5 ng/mL (174).

Methods to determine APAP and metabolites in human urine have been presented with detection by ultraviolet or electrochemical means. Knox and Jurand studied the effect of negative and positive pairing ions on retention of APAP and the sulfate, glucuronide, cysteine and mercapturic acid metabolites on ODS and end-capped ODS columns with UV detection (175). In addition, salt effect in mobile phase and equilibration of ion-pairing agent with the column were examined. A second urinary metabolite direct injection method was described which related metabolite content to enzyme stimulation and inhibition in rodent species (176). The reversed-phase separation of APAP and 14 metabolites from urine was studied by applying radial compression and traditional 30 cm columns with UV detection (177). A gradient program from 10-50% methanol afforded 12 minute run times using a tetrabutylammonium pairing ion and permitted the separation of phenacetin as well.

The determination of APAP and metabolites in human urine by HPLC with electrochemical detection has further extended detection limits. An oxidation potential of 1.1 v was applied over a carbon paste electrode to measure APAP and six metabolites from urine amperometrically (178). Detection limits for the metabolites and APAP were in the picogram range with a recovery of 23.8% of the total dose excreted in the urine in 4 hours. Similar amperometric

detection limits were observed for APAP and metabolites in a study which compared UV detection at 248 nm to electrochemical detection at 0.55 v (179). Figure 8 shows the results of a β-glucuronidase-sulfatase hydrolysis experiment on urine samples indicating the increased sensitivity and selectivity of EC detection.

Pharmacokinetics of acetaminophen in humans have been measured by HPLC with electrochemical or ultraviolet detection. An early electrochemical method with a carbon paste electrode determined the plasma half-life to be 2.2 hours (180). Three dosage forms were compared using ethyl acetate plasma extractions and finding linearity between 0.2 and 4.0 µg/mL. Another study used UV detection and a similar ethyl acetate extraction procedure (181). Two mobile phases were shown to be equally effective in a study comparing solution and tablet APAP dosage forms (182). A limit of detection of 0.1 µg/mL in plasma was shown with determinations of absorption and elimination half-lives, total clearance, bioavailability and the hepatic extraction ratio. Recoveries of 101.5% at 0.5 µg/mL and 99.8% at 1.0 µg/mL were found in another study using acetonitrile as extraction solvent (183). A tablet containing acetaminophen and pentazocine (650 and 25 mg respectively) was compared to a solution dosage form in the same proportion in a human study using a phenyl column for the plasma APAP assay (184). Parameters were calculated using a weighted nonlinear regression comparing model independent and regression dependent methods.

Acetaminophen, phenacetin and metabolites have been separated by HPLC from animal fluids and measured

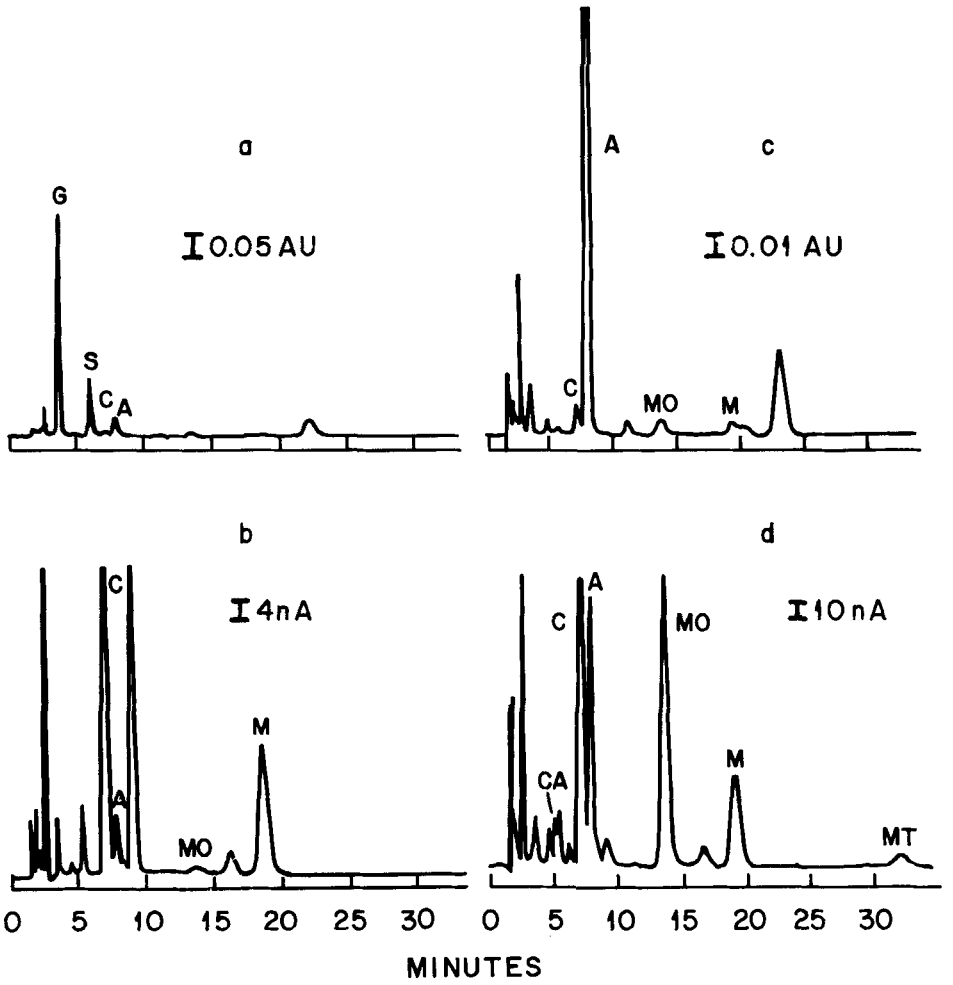


FIGURE 8. (a) Chromatogram (UV detection, 248 nm) of a 10 µL sample of a urine aliquot diluted 1:4 with 2 M acetate buffer, pH 5.0. Based on standard curves and recovery data, estimated amounts of acetaminophen glucuronide (G), acetaminophen sulfate (S), and free acetaminophen (A) are 304 µg/mL, 112 µg/mL and 23 µg/mL respectively. (b) Chromatogram (electrochemical detection, +0.55 v) of the same eluent indicating the presence of the cysteine conjugate (C), free acetaminophen (A), free methoxyacetaminophen (MO) and acetaminophen-3-mercapturate (M). (c) Chromatogram (UV detection, 248 nm) of a 10 µL sample of a second aliquot of the patient's urine after β-glucuronidase-sulfatase hydrolysis. (d) Chromatogram (electrochemical detection, + 0.55 v) of the same eluent. Based on standard curves and recovery data, the hydrolyzed sample contained 3-hydroxyacetaminophen (CA, 0.43 µg/mL), 3-cysteinylacetaminophen (C, 30 µg/mL), acetaminophen (A, >400 µg/mL), 3-methoxyacetaminophen (MO, 78 µg/mL), acetaminophen-3-mercapturate (M, ca. 91 µg/mL), and 3-methylthioacetaminophen (MT, 11 µg/mL). Reprinted with permission Elsevier Science Publishers and authors.

by ultraviolet or electrochemical detection. A method was demonstrated for acetaminophen and phenacetin determination in rat urine with calculations made by means of a radioactive internal standard (185). The collection of eluent fractions after passing through the UV detector followed by liquid scintillation counting was necessary here. The involvement of minor APAP metabolites in animals related to possible toxic reaction mechanisms was studied by two groups. Kissinger and Miner used LCEC to provide evidence that N-acetyl-p-quinoneimine was a toxic intermediate in mouse liver microsome metabolism of APAP (186). Further the reaction of this intermediate with sulfhydryl containing nucleophiles in microsomes was studied using both neutral and acidic mobile phases. The same intermediate was implicated in a comprehensive study of minor metabolites found in hamster urine (187). Five different mobile phases were used for the identification of nine metabolites from diluted and filtered 24 hour urine samples. Hart and coworkers provided two different reversed-phase methods for the separation of APAP metabolites in rat urine: ion suppression and ion-pairing (188). The effect of mobile phase pH in the former and isocratic or gradient programmed elution in the latter methods were related to metabolite separations. APAP and major metabolites in rat plasma and urine were measured using a rapid, micro sample preparation method and reversed-phase chromatography (189). Perchloric acid precipitated proteins to give 92.2% APAP recovery.

Kinetics studies employing HPLC assays on acetaminophen and metabolites have been reported in

TABLE 3A

HPLC CONDITIONS FOR ANALGESIC ASSAYS

p-Aminophenol Derivatives-Biological Studies

Column	Mobile Phase	Detection	Linearity	Reference
25 cm silica 5-6μ	water saturated chloroform:heptane: ethanol:acetic acid (540:360:100:0.72)	UV/254nm	4-100 μg/mL	168
30 cm C_{18} 10μ	acetonitrile:water:acetic acid:triethyl-amine (250:2750:1.5:2)	UV/204nm	1-80 μg/mL	169
30 cm C_{18} 10μ	methanol:water:acetic acid(300:700:0.7)	UV/250nm	-	170
15 cm C_8 5μ	methanol:0.0025 M NaH_2PO_4 pH 6.6 with 0.065% triethylamine (14:86)	UV/270nm	11-150 μg/mL	171
30 cm C_{18} 10μ	water:1 M ammonium acetate:methanol: 1 M acetic acid (390:34:70:9)	EC/0.65v	20 ng/mL-20 μg/mL	172
C_{18}	0.1 M sodium phosphate pH 4.9:methanol (96:4)	EC/0.75v	-	173
25 cm C_{18} 5μ	sodium perchlorate:sodium citrate:meth-anol:water (28.1 g:1.5 g :130:870)pH 5.0	EC/0.8v and UV/254nm	2-200 ng injected	174
12.5 cm ODS 7μ	water:methanol:formic acid (86:14:1) with dioctylamine (7 mg/L) or tetrabutylam-monium hydroxide (200 mg/L)	UV/242nm	-	175
30 cm C_{18} 10μ	acetonitrile:0.02 M phosphoric acid (2.5:97.5) pH 4.7	UV/254nm	0.1-10 mmol/L	176
radial compression C_{18} 10μ or 30 cm C_{18} 10μ	A) methanol:0.05 M potassium phosphate pH 2.3 (15:85) with triethylamine (0.005 M) or B) tetrabutylammonium hydroxide (0.005 M or 0.01 M) in methanol:water (1:1) or with 0.005 M EDTA	UV/254 and 280nm	-	177

25 cm ODS 5µ	methanol:0.1 M acetate buffer pH 4.0 (3.5:96.5)	EC/1.1v	-	178
30 cm C_{18} 10µ	methanol:acetic acid:0.1 M KH_2PO_4 (7:0.75:92)	UV/248nm and EC/.55v	0.42-42 µg/mL	179
50 cm polyamide 37µ	0.04 M NaH_2PO_4 pH 7.4:methanol (95:5)	EC/0.7v	-	180
25 cm RP-8 10µ	water:acetonitrile (94:6)	UV/254nm	0.5-20 µg/mL	181
30 cm C_{18} 10µ	acetonitrile:acetate buffer pH 4.0 (3.5:96.5) or acetonitrile:methanol: water (6:6:88)	UV/254nm	0.25-15 µg/mL	182
25 cm ODS 5µ	methanol:water (25:75)	UV/254nm	0.1-20 µg/mL	183
30 cm phenyl 10µ	acetonitrile:0.1 M KH_2PO_4 pH 2.4 (7:93)	UV/254nm	0.1-15 µg/mL	184
30 cm C_{18} 10µ	acetonitrile:water:PIC-B-7 (30:70:1.6)	UV/254 and 280nm and liquid scint.	50-5000 µg/mL	185
15 cm C_{18} 10µ	1 M acetic acid:1 M ammonium acetate: methanol:water (10:35:25:400)	EC/0.75v	-	186
30 cm C_{18} 10µ or 25 cm C_{18} 5µ	dioxane:acetic acid:0.005 M heptane-sulfonate (5:1:94) or methanol:acetic acid:3.5 mM heptanesulfonate (5:1:93) or dioxane:acetic acid:10mM pentane-sulfonate (6:1:93) or acetonitrile:10 mM NaH_2PO_4(pH7)(12:88) or the latter (17:83)	UV/254nm	-	187
30 cm C_{18} 10µ	1) 1% acetic acid:methanol:ethyl acetate (900:150:1) or 2) methanol:0.05 M phosphoric acid (15:85) pH 2.8 or 3) 0.005 M pH 7.2 tetrabutylammonium phosphate (TBA) or 4) gradient-A) 0.005 M TBA, 0.01 M Tris, 0.005 M EDTA, pH 7.2, B) A:methanol (1:1)	UV/254nm and liquid scintillation	-	188

(continued)

TABLE 3A (continued)

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Column		Mobile Phase	Detection	Linearity	Reference
25 cm C_{18}	5µ	acetonitrile:0.05 M sodium sulfate pH 2.2 (7:93)	UV/254nm	5-200 µg/mL	189
30 cm C_{18}	10µ	gradient-A) 1% acetic acid, B) 1% acetic acid:methanol:ethyl acetate (90:15:0.1)	UV/250nm	-	190
25 cm C_{18}		methanol:0.01 M ammonium phosphate and 0.1 M trichloroacetic acid pH 2.7 (10:90)	UV/254nm and EC/0.55v	-	191
30 cm C_{18}	10µ	methanol:water (30:70) or methanol:0.05 M KH_2PO_4 and 0.75% acetic acid (13:87)	UV/254nm	-	192
25 cm ODS	5-6µ	0.005 M NaH_2PO_4 and 0.12 M tetrabutylammonium hydroxide pH 3.0:methanol (75:25)	UV/250nm	13.2-6615 µM	193
25 cm C-18	5µ	0.05 M sodium sulfate:acetonitrile (30:50) pH 2.2	UV/254nm	-	194

several species and in vitro experiments. The rates of glucuronide, sulfate, glutathione and cysteine conjugation of APAP in isolated rat and mouse hepatocytes were investigated using a reversed-phase gradient elution system (190). A method to calibrate in vivo electrodes by means of standard acetaminophen i.p. injection in rats was proposed in order to study brain catechol levels by amperometry (191). Kinetics of serum and caudate nucleus levels of APAP were determined with UV detection and related to in vivo EC detector response at 0.55 v. The relative rates of sulfation and glucuronidation of APAP by perfused rat liver were studied by HPLC (192). Blood was assayed following ethyl acetate extractions and bile after dilution using standards from 0.1-45 µg carried through the procedure. Acetaminophen pharmacokinetics in rats were studied with unchanged drug and major metabolite determinations in plasma, bile and urine made by HPLC (193). While a two-compartment model fit the data in the above study, a one-compartment model was used to describe pharmacokinetics of APAP in rats under varying conditions of protein nutrition (194). Plasma and urine samples were assayed by reversed-phase HPLC following acetonitrile protein precipitation and dilution respectively.

Dosage Form Studies

Literature covering the HPLC analysis of acetaminophen and phenacetin alone in dosage forms has been of a very limited nature. In this area a normal phase method was proposed to assay APAP in capsules, tablets, elixirs, suspensions and suppositories (195). Tablet and capsule samples were prepared by sonicating in a water-methanol mixture, filtering, adding

internal standard and chloroform while elixirs, suspensions and suppositories were dissolved in methanol or chloroform-methanol. An ion-pairing method with a phenyl column was developed for acetaminophen in effervescent tablets (196). An average recovery from spiked placebos of 100.8% was found with the ability to separate p-aminophenol from APAP by the method. Phase rearrangement in which the C_8 groups of an octyl column were extended by heating at 55° was used to give shorter retention times for APAP and make possible use of water alone as the mobile phase (197). Individual tablets, capsules and composite tablet mixtures were assayed by this method where samples were taken up and diluted in water. A normal phase assay of impurities in bulk phenacetin powder was described with detection by UV at 247 nm (198). Acetanilide, p-chloroacetanilide and p-phenetidine were assayed by standard addition to pure phenacetin in the hundreds of ppm range.

Several methods for the analysis of acetaminophen in combination products in addition to those discussed in the aspirin section above have been published. One such method gave mean recoveries of 101.5% and 100.6% for APAP in synthetic cough mixtures and commercial preparations respectively (199). In this method it was possible to assay guaifenesin, pseudoephedrine, pholcodine and parabens simultaneously with APAP. An official method provides for an APAP assay, a salicylic acid assay and an APAP dissolution test for APAP and aspirin tablets to be run by reversed-phase HPLC (200). Another procedure has been described for APAP in a combination tablet with caffeine and propylphenazone which employed

TABLE 38

HPLC CONDITIONS FOR ANALGESIC ASSAYS

p-Aminophenol Derivatives-Dosage Form Studies

Column	Mobile Phase	Detection	Linearity	Reference
30 cm silica 10µ	butyl chloride:water saturated butyl chloride:THF:methanol:acetic acid (475:475:70:35:30)	UV/254nm	72-122% label claim	195
30 cm phenyl 10µ	0.005 M tetrabutylammonium phosphate: acetonitrile (85:15) pH 7.5	UV/254nm	0.01-0.03 mg/mL	196
5 cm C_8 5µ	water	UV/254nm	30-130 µg/mL	197
10 cm silica 5µ	hexane:ethyl acetate (1:1)	UV/247nm	0.3-.5 mg/mL	198
30 cm C_{18} 10µ	methanol:water:acetic acid (45:55:2) 0.005 M octanesulfonate	UV/254 and 280nm	80-120% label claim	199
30 cm C_{18}	tetrabutylammonium hydroxide:water: methanol:acetonitrile:acetic acid (225 mg:750:125:125:1)	UV/280nm	-	200
25 cm C_{18} 10µ	gradient-methanol:water (30:70) to (95:5)	UV/270nm	0.125-1 mg/mL	201
30 cm phenyl 10µ	1) 0.02 M ammonium acetate pH 7: methanol (85:15) or 2) 0.02 M KH_2PO_4 pH 2.6 or 3) 0.005 M heptanesulfonate: acetic acid:methanol (51:1:48) or 4) 1% ammonium formate pH 4.1:methanol (60:40)	UV/262 and 279nm	-	202
25 cm RP-8 10µ	methanol:0.2 M phosphate buffer pH 3.5:water (20:10:70)	UV/254 and 280nm	-	203
30 cm C_{18} 10µ *or* 30 cm cyano 10µ	methanol:0.025 M NaH_2PO_4 pH 7.0 (10:90) to (70:30)	UV/254 and 280nm	-	204
25 cm C_8 6µ	0.1 M citrate buffer pH 4.0:methanol (86:14)	EC/0.66v	-	205
25 cm C_{18}	acetonitrile:water (90:10)	UV/diode array	-	206

gradient elution (201). Mean recoveries of 100.2% each were found for APAP in synthetic mixtures and in tablet formulations run at 0.5 mg/mL concentration. Acetaminophen was measured in tablets and elixirs in combination with chlorpheniramine, dextromethorphan and phenylpropanolamine by using four different mobile phases with separate sample dilutions for each active (202). A 100.8% recovery for APAP was measured from synthetic mixtures. Acetaminophen was analyzed simultaneously with aspirin, ascorbic acid and several degradation products in tablets using a method with UV detection at 254 nm (203). The stability of each active in samples aged to 61 months and stressed at 37° and 50° were measured following initial dissolution in formic acid-methanol.

Several miscellaneous studies involving HPLC analysis of acetaminophen or phenacetin have appeared in print. J.K. Baker described a retention index system for a series of compounds including phenacetin prior to its application to opiates as described above (204). Retention on octadecylsilane and cyano columns was examined along with mobile phase changes in this study. The effect of temperature on the electrochemical detection of acetaminophen has been described as due to diffusion and heterogeneous electron transfer (205). Variation due to temperature fluctuations could be minimized according to the author by controlling the system or column temperature, increasing the flow rate, operating at the limiting current region or by proper choice of internal standard. Finally acetaminophen was included in a study demonstrating the use of a UV photodiode array detec-

tor in observing unresolved peaks (206). It was found that APAP and benfotiamine, with similar UV spectra, could each be quantitated even when their chromatographic peaks were artificially superimposed.

C. Arylacetic and Arylpropionic Acid Derivatives

The arylacetic acids are a more recently developed class of nonsteroidal anti-inflammatory analgesic drugs. Major interest in this group has centered on ibuprofen, naproxen and zomepirac regarding publication of pharmacokinetics and metabolic studies which require chromatographic separation. Little work other than biological studies for these compounds has been published as will be seen below.

Ibuprofen

Ibuprofen, one of the more popular prescription and nonprescription NSAIDs with analgesic properties, has been studied in both man and animals with respect to metabolism and pharmacokinetics using HPLC methods. One report described a method for extracting ibuprofen from human or dog plasma with methylene chloride following protein precipitation with 5 N HCl (207). Recoveries of 82.5% and 100.0% were found at 50.0 µg/mL and 6.25 µg/mL levels respectively and selectivity was shown by the separation of ibuprofen from phenylbutazone, salicylamide, phenacetin and APAP. Another method to determine ibuprofen in human plasma was demonstrated using fluorescence detection (208). An overall recovery of 74.9% was found using a hexane extraction. Ethyl acetate was used for ibuprofen extraction from human serum in another study which gave 95% recovery (209).

Pharmacokinetics of ibuprofen in humans were studied using reversed-phase HPLC with UV detection in all cases. Wagner and coworkers used two separate systems, one a gradient elution method to quantitate unchanged drug and metabolites in urine, with and without basic hydrolysis, and a second, an isocratic system to measure ibuprofen in plasma (210). A chromatogram of a typical urine extract is shown in Figure 9. Extractions in each case were performed with methylene chloride and recoveries of 93.7-107.6% were found for ibuprofen in urine. A recovery of 57.2% was found in a study extracting ibuprofen from human plasma with pentane-ether followed by 1.0 M NaOH and reextraction of acidified samples with pentane-ether (211). Another determination of human plasma and cat CSF ibuprofen levels was made with chromatography on a C-18 radial compression column (212). A benzene-isoamyl alcohol extraction was carried out on acidified samples. A second group using a C-18 radial compression column investigated the extraction effeciency of 6 solvents for ibuprofen from acidified human plasma (213). The highest recovery (102.6%) was found using isooctane-2-propanol. The lack of effect of concurrent cimetidine therapy on ibuprofen pharmacokinetics was found using reversed-phase chromatography with detection at 220 nm (214).

Kinetics studies on ibuprofen in animals have also been undertaken using HPLC. Plasma levels in rabbits following administration of suppositories made from three different bases were measured (215). Linearity between 0.1 and 50 µg/mL was found for this nonextraction method. Rabbit myocardial pharm-

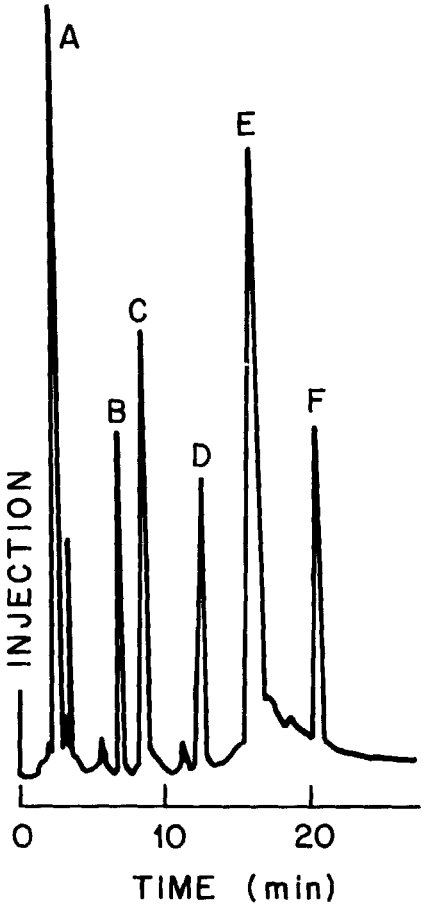


FIGURE 9. Typical chromatogram of ibuprofen and its metabolites extracted from urine. A = solvent front and highly polar contaminants; B = OH-ibuprofen; C = COOH-ibuprofen; D = methylprednisolone; E = low polar contaminants; F = ibuprofen. Reprinted with permission Elsevier Science Publishers and authors.

acokinetics were investigated by determining ibuprofen levels in perfusion fluid and finding that a two-compartment model gave best fit to the results (216).

Chromatographic separation of enantiomers of ibuprofen has been accomplished by either forming a diastereomeric product with a base or by use of a chiral stationaly phase (Pirkle) column. In the first method amide derivatives of ibuprofen and naproxen with 1-phenylethylamine prepared from an imidazolide intermediate were chromatographed on a silica column (217). Here the (R-acid;S-amine) diastereomer eluted before the (S-acid;S-amine) as was also seen in a biological study of ibuprofen enantiomer pharmacokinetics (218). The latter study used S(+)-2-octylester derivatives of the acid to show that an in vivo stereoselective inversion of the R(-) to the S(+) enantiomer occurs. Chromatography of ibuprofen-1-naphthalenemethylamide optical isomers on a chiral stationary phase (CSP) resulted in the elution order: (S) enantiomer first and (R) enantiomer second (219, 220). This was due to the stability of bonds formed to the 3,5-dinitrobenzoylphenylglycine bonded to the silica gel which gave less satisfactory separation of chiral acid esters.

Two methods have described the simultaneous assay of ibuprofen with several other analgesic NSAIDs in serum or plasma by HPLC. One procedure involved an acetonitrile precipitation step and mobile phases buffered at pH 3.0 or 7.8 (221). Ibuprofen, naproxen, phenylbutazone and indomethacin among others were separated with recoveries between 80 and 102%. A chromatogram of the resulting separation is shown in Figure 10. A later method was

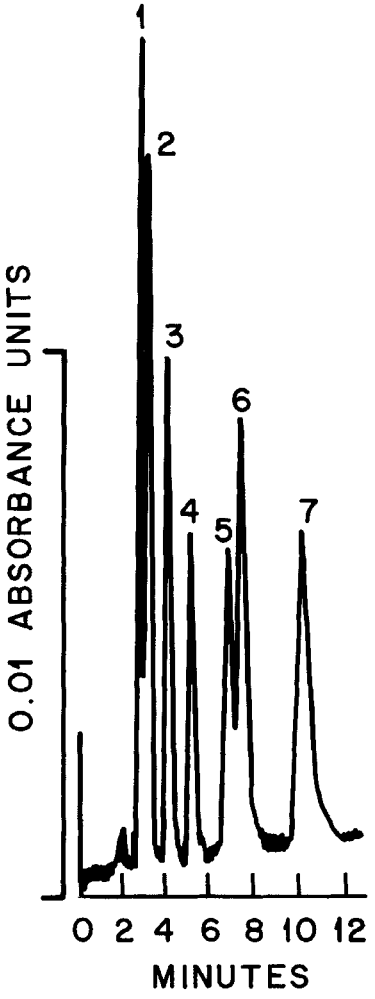


FIGURE 10. Separation of anti-inflammatory drugs by HPLC using 35% acetonitrile in 0.7% NH_4Cl buffered to pH 7.8 at a flow rate of 1.0 mL/min and wavelength 225 nm. Peaks: 1 = oxyphenbutazone (100 ng); 2 = naproxen (5 ng); 3 = phenylbutazone (100 ng); 4 = ibuprofen (200 ng); 5 = indomethacin (50 ng); 6 = mefenamic acid (100 ng); 7 = flufenamic acid (100 ng). Reprinted with permission Elsevier Science Publishers and authors.

developed which required a methylene chloride extraction of acidified serum or blood samples (222). Ibuprofen, naproxen, fenprofen, indomethacin, phenylbutazone and others were determined at 240 nm.

Few studies have appeared on HPLC separations of arylacetic and arylpropionic acid NSAIDs which assay the bulk drugs or dosage forms. One such study showed that an ion-suppression mechanism gave recoveries from 99.5-102.0%, 100.0-100.6%, 99.0-101.6% and 100.0-101.3% for ibuprofen in tablet composites, bulk drug, single tablets and synthetic mixtures respectively (223). The proposed method was more rapid, simpler and gave more accurate and precise recoveries than the current USP g.c. method.

Naproxen

The second of the arylpropionic acid analgesics included here has been determined in serum, plasma and urine by HPLC, usually in combination with other NSAIDs. Plasma and urinary concentration-time profiles were examined in two studies using UV detection (224, 225). The first employed an ether extraction of plasma or urine giving 90 and 80% recovery respectively, although a column life of 300 injections was found for ketoprofen run by the same method. The second used a chlororform extraction since an unknown peak was observed in chromatograms at 40 minutes following ether extractions. Mixed aqueous-organic mobile phases were studied at different pH values and buffer concentrations to make possible the separation of 10 other NSAIDs. A method was developed for the simultaneous assay of naproxen and salicylic acid in serum which compared UV detection at 235 nm and 254 nm to fluorescence detection (226). A rapid

TABLE 4A

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Arylacetic Acids

Column		Mobile Phase	Detection	Linearity	Reference
ibuprofen					
30 cm C_{18}	10μ	methanol:water:acetic acid (75:24:1) pH 3.4	UV/272nm	0.5-100 μg/mL	207
25 cm ODS	6μ	acetate buffer (0.1 M pH 5):methanol (35:65)	fluorescence 253/420nm	1-40 μg/mL	208
10 cm C_{18}	3μ	acetate buffer (0.1 M pH 6.4):acetonitrile (65:35)	UV/220nm	1-100 μg/mL	209
25 cm ODS-3	10μ	1) gradient-A) acetonitrile:water:phosphoric acid:acetone (280:720:0.5:0.5) B) acetonitrile:KH_2PO_4 0.05 M (50:50) or 2) methanol:water:phosphoric acid (700:300:1)	UV/210nm	1-140 μg/mL	210
15 cm ODS	6μ	acetonitrile:water:phosphoric acid (550:450:0.5)	UV/195nm	1-100 μg/mL	211
10 cm radial compression C_{18}	10μ	water:acetonitrile:acetic acid (500:500:2.5)	UV/229nm	1-50 μg/mL	212
10 cm radial compression C_{18}	10μ	water:acetonitrile:phosphoric acid (500:500:1)	UV/220nm	0.2-60 μg/mL	213
25 cm ODS	5μ	methanol:water:phosphoric acid (800:200:1)	UV/220nm	1-70 μg/mL	214
30 cm C_{18}	10μ	acetonitrile:0.1 M acetic acid (55:45)	UV/254nm	0.1-50 μg/mL	215
30 cm C_{18}	10μ	methanol:0.05 M phosphate buffer pH 4 (60:40)	UV/214nm	-	216
25 cm silica	10μ	isopropanol:cyclohexane (7:93)	UV/225nm	-	217
25 cm silica	5μ	isopropanol:hexane (0.05:99.95)	UV/220nm	0.5-60 μg/mL	218

(continued)

TABLE 4A (continued)

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Column	Mobile Phase	Detection	Linearity	Reference
25 cm Pirkle covalent 5µ	isopropanol:hexane (1:99)	UV/254nm	-	219
30 cm C_{18} 10µ	acetonitrile:0.045 M KH_2PO_4 pH 3 or pH 7.8 (60:40)	UV/225nm	5-80 µg/mL	221
15 cm ODS 5µ	acetonitrile:water:acetic acid (450:550:3.2)	UV/240nm	10-400 µg/mL	222
15 cm ODS 5µ	acetonitrile:0.25 M acetic acid (75:25)	UV/254nm	62.8-2010 µg/mL	223
naproxen				
4 cm ODS 5µ	0.05 M Na_2HPO_4-KH_2PO_4 buffer pH 7.0: acetonitrile (92:8)	UV/262nm	200ng/mL-10µg/mL	224
15 cm ODS 5µ	acetonitrile:aqueous phosphoric acid pH 3 (45:55)	UV/230nm	1-120 µg/mL	225
30 cm C_{18} 10µ	methanol:0.1 M acetic acid (70:30)	UV/254 and 235nm and fluorescence 240/340nm	1-100 µg/mL	226

25 cm RP_{18}	10µ	acetonitrile:0.05 M KH_2PO_4 pH 3.7 (55:45)	UV/254nm	1-100 µg/mL	227
10 cm RP_2	6µ	phosphate buffer pH 7:methanol (7:3) or acetate buffer pH 4.8:methanol (1:1)	UV/261nm	-	228
or					
10 cm RP_8	6µ	phosphate buffer pH 6:methanol (1:1)			
or					
10 cm C_8	5µ	as above			
zomepirac					
25 cm silica	10µ	hexane:isopropanol:acetic acid (93:2:5)	UV/313nm	10-5000 ng/mL	229
25 cm ODS	10µ	methanol:0.01 M acetate buffer pH 4.0 (45:55)	UV/254nm	4-1000 µg/mL	230
30 cm C_{18}	10µ	0.1 M KH_2PO_4 pH 4:acetonitrile (60:40)	UV/330nm	0.1-10 µg/mL	231
25 cm ODS	10µ	methanol:0.01 M phosphate buffer pH 3 (45:55)	UV/313nm	-	232
15 cm ODS	5µ	methanol:0.01 M tetrabutylammonium hydrogen sulfate and 0.05 M sodium acetate pH 4.5 (58:42)	UV/313nm	0.1-10 µg/mL	233
12.5 cm ODS	5µ	acetonitrile:water:phosphoric acid (50:49.5:0.5)	UV/313nm	0.5-100 µg/mL	234

acetonitrile protein precipitation method was found useful for microscale determination of plasma naproxen (227). While the limit of detection was 0.5 µg/mL the demethylated metabolite could not be quantitated in the same chromatogram due to interference from an endogenous component. Plasma protein binding of naproxen was investigated in a study in which plasma samples were only centrifuged, filtered and diluted before injection (228). Naproxen peak deformation was examined as a function of the binding equilibrium by dilution and displacement techniques with four different reversed-phase systems.

Zomepirac

The arylacetic acid analgesic, zomepirac, has been measured in biological fluids by HPLC. Plasma levels were determined using a normal phase method and an isoamyl alcohol-heptane extraction (229). Recoveries of 78.3-83.0% were found from spiked plasma samples. Urinary drug and glucuronide conjugate levels were obtained by a non-extraction reversed-phase procedure (230). The glucuronide was hydrolyzed with 6 N NaOH in one hour and recoveries from 96.3-102.6% were demonstrated. Plasma kinetics of zomepirac were followed in three studies using normal or reversed-phase methods. Detection at 330 nm was used in the first with a mobile phase buffered at pH 4.0 and an ether extraction of acidified plasma (231). This study found plasma elimination half-lives from 1.5-2.2 hours. While the second study measured plasma and urine levels to determine clearance values when aspirin was administered concomitantly (232), the instability of zomepirac glucuronide was examined in the third

study where a tetrabutylammonium ion-pairing system was used (233). Rapid hydrolysis and acyl migration in the conjugate required special sample handling techniques including use of a refrigerated centrifuge and prebuffering of sample vials. A column-switching approach was used to enable fully automated HPLC of plasma and urine samples with initial partition of the drug onto an ODS precolumn by another group (234). Following this treatment a mixed organic mobile phase eluted the drug in the same direction from the precolumn to the analytical column where recoveries of 95.1-96.9% were found.

D. Pyrazole Derivatives

The pyrazole anti-inflammatory analgesics including the pyrazolone antipyrine and the pyrazolidinedione phenylbutazone are not new drugs. While their use has been somewhat limited by unpleasant side effects they have been studied predominately in the biological area with analysis carried out by HPLC.

Phenylbutazone

Phenylbutazone, usually in combination with its metabolites oxyphenbutazone and hydroxyphenylbutazone, have been determined in fluid matrices of both humans and animals. All reports have included ultraviolet detection. One human study revealed a separation of the three components and an internal standard in 16 minutes run time following a rapid acetonitrile precipitation of plasma protein (235). Pharmacokinetics in man have been delineated in three reports. A normal phase method separated phenylbutazone from oxyphenbutazone in human plasma after

TABLE 4B

HPLC CONDITIONS FOR ANALGESIC ASSAYS

Pyrazole Derivatives

Column		Mobile Phase	Detection	Linearity	Reference
phenylbutazone					
10 cm ODS	5μ	methanol:acetonitrile:1% acetate buffer pH 3.5 (26:20:54)	UV/240nm	10-200 μg/mL	235
25 cm silica	7μ	hexane:THF:acetic acid (780:220:1)	UV/239nm	1-75 μg/mL	236
25 cm RP-18	10μ	methanol:phosphate buffer pH 4 (63:37)	UV/254nm	0.154-154 μmol/L	237
4 cm RP-8	10μ	acetonitrile:water (1:1) pH 3.2 with phosphoric acid	UV/254nm	0.5-50 μg/mL	238
25 cm C_{18}	10μ	acetic acid:water:methanol (2:35:65)	UV	-	239
15 cm RP-18	5μ	0.02 M phosphate buffer pH 7.0:methanol (550:450)	UV/254nm	0.1-1.5 μg/mL	240
30 cm C_{18}	10μ	gradient-A) methanol:0.01 M acetate buffer pH 4 (50:50) B) 100% methanol	UV/254nm	0.5-50 μg/mL	241
30 cm C_{18}	10μ	methanol:0.05% acetic acid (65:35)	UV/254nm	4-40 μg/mL	242

antipyrine					
25 cm RP-2	10µ	gradient-A) 0.05 M phosphate buffer pH 6.5:methanol (28:72) B) methanol or phosphate buffer (pH 6.5 0.05 M): methanol (60:40)	UV/254nm	0.1-20 µg/mL	243
10 cm MOS(C_8)	5µ	0.02 M phosphate buffer pH 7.2:acetonitrile (100:10) with sodium pyrosulfite 2 g/L or 0.0067 M phosphate buffer pH 7.2 :acetonitrile (100:8)	UV/254nm	10-200 µg/mL	244
30 cm C_{18}	10µ	methanol:0.067 M phosphate buffer pH 3.5 (35:65)	UV/254 and 225nm	1.25-100 µg/mL	245
30 cm C_{18}	10µ	gradient-A) acetonitrile:water (17:83) B) acetonitrile:water (1:1) C)acetonitrile	UV/254nm and liq. scint.	-	246
10 cm RP-2	5µ	acetonitrile:phosphate buffer (pH 6.5 0.05 M) (5:95)	UV/244nm	0.1-20 µg/mL	247
30 cm C_{18}	10µ	acetonitrile:1% acetic acid (35:65)	UV/254nm	1-80 µg/mL	248
10 cm C_8 radial compression	10µ	methanol:0.15 M ammonium acetate (33.5:66.5)	UV/252nm	2.6-40.8 µg/mL	249
30 cm C_{18}	10µ	methanol:water (50:50)	UV/254nm	1.5-50 µg/mL	250
10 cm SAS(C_1)	5µ	methanol:0.05 M pH 7 phosphate buffer (25:75)	UV/270nm	1-50 µg/mL	251
25 cm cyano		0.02 M phosphate buffer:acetonitrile:triethylamine (90.5:9:0.5) pH 4.0	UV/244nm	-	252
30 cm C_{18}	10µ	acetonitrile:water:acetic acid (32.5:67.5:0.5)	UV/254nm	0.223-2.23 µg/mL	253
15 cm silica	5µ	chloroform:methanol (97.5:2.5)	UV/275nm	-	254

a chloroform-diisopropyl ether extraction (236). A detection limit of 0.5 µg/mL was found with detection at 239 nm. A toluene extraction of plasma in another study gave recoveries from 92-108.5% between 0.154 and 154 µmol/L (237). This method was applied to a suppository dosage form while the final human study was conducted on drug administered by the intramuscular route (238). A cyclohexane-ether solvent mixture was used as extractant to give plasma levels measured at 254 nm with 88.4% recovery at 2 µg/mL.

Phenylbutazone in animal fluids has been measured using reversed-phase methods both with and without pharmacokinetics measurements. In the latter group, the drug was extracted from horse plasma, urine or sweat with hexane in a study which found 65% recovery (239). The difficulty of assaying bovine milk for phenylbutazone was pointed out in a study which accomplished this goal with 89% recovery at 100 ng/mL (240). Hexane was used here too for extractions with the standard curve running from 0.1-1.5 µg/mL. Pharmacokinetics were measured in the rat and dog with plasma and urine samples assayed at 254 nm (241). A linear gradient mobile phase on a C_{18} column gave recoveries of 96.7, 93.1 and 81.7% for phenylbutazone, oxyphenbutazone and hydroxyphenylbutazone respectively. A recent study of phenylbutazone kinetics in the rabbit was carried out on a C_{18} column at 254 nm (242). Models were developed for oral, intravenous and percutaneous drug administration with simultaneous measurement of oxyphenbutazone.

Antipyrine

Chromatography of antipyrine, the analgesic NSAID which is also used in a liver enzyme function

test, has been carried out on human and animal fluid and tissue samples. Pharmacokinetics measurements have been made in certain cases. Determinations of antipytine and metabolites in plasma, urine and saliva were made using C-2 (243) and C-8 (244) columns. Dichloromethane and dichloromethane-pentane were used for extraction of the drug and metabolites from urine or plasma in these procedures. An octadecylsilane column was used in a third study which only measured antipyrine levels in human serum (245). The drug in human or rat liver biopsy samples was determined following a toluene extraction using UV and liquid scintillation detection (246). Metabolites were isolated by ethyl acetate and dichloromethane extractions. Human pharmacokinetics studies were all done on reversed-phase columns. One method employed an RP-2 column and related k' values to mobile phase pH and % acetonitrile (247). While a second method used a C-18 column (248), a third study used a radial compression C_8 column for antipyrine and metabolite separations having found C-2, C-8 and C-18 columns less effective (249). In this study the difference in pharmacokinetics between smokers and nonsmokers was examined.

Pharmacokinetics of antipyrine have also been investigated in the rat and rabbit using HPLC. Plasma levels were measured in one study following a $ZnSO_4$ sample treatment using a C_{18} column (250). A C-1 SAS column was used in a second study in which acetonitrile precipitated rat plasma protein (251). Urinary antipyrine and metabolites were measured on the same column following a chloroform extraction. The effect of trauma on rat liver antipyrine metabol-

ism was investigated utilizing a cyano column to determine plasma antipyrine levels (252). Pharmacokinetics of antipyrine in the rabbit were also studied measuring plasma levels on a C_{18} column after dichloromethane extraction (253). A recovery of 104% was found at a plasma level of 2.23 µg/mL.

A nonbiological investigation on antipyrine normal phase chromatographic behavior was carried out in an attempt to determine the effect of injecting samples in a noneluting solvent (254). Solutions of antipyrine and phenacetin in n-pentane-methanol (97.5:2.5) gave minimum peak widths as the injection volume increased. The slope of the plot of retention volume vs. volume of sample injected was used to show that the optimized solvent was neither an eluting or a noneluting solvent.

CONCLUSION

The rapid expansion of high-performance liquid chromatographic separations in the field of analgesic analysis has been fundamental to many of the concurrent advances in drug disposition, dosage form analysis and forensic sciences. In each of these fields HPLC has given its powers of specificity, accuracy, precision and speed to solve problems previously without solution or where imprecise or incorrect measurements were the only ones available.

It should be obvious from the tables included here which research areas should come under future investigation in analgesic analysis by HPLC. Work in the area of dosage form analysis has in general made a poor showing relative to biological studies both human and animal. In each area novel detection

methods will continue to extend minimum quantifiable levels below their present limits. The application of electrochemical detection to biological studies of analgesics is an example of this. Further work in drug derivatization to enhance detectability and chromatographic system selectivity is needed. Chiral separations will become increasingly important in view of activity differences between (R) and (S) isomers already seen. The development of new non-silica based columns could enhance separations and open the entire mobile phase pH range rather than one-half of it to investigation. Automation in sample preparation and chromatographic instrumentation as well as data acquisition will continue to make progress more rapid. The current abundance of high quality commercial HPLC instrumentation will continue to have a major impact on drug regulatory requirements and official methods of analysis whose parallel growth has occurred in the past.

REFERENCES

1. Fishbein, L., Chromatography of Environmental Hazards, Volume IV, Drugs of Abuse, Elsevier, Amsterdam, 1982.

2. Gough, T.A. and Baker, P.B., J. Chromatogr. Sci., 20, 289 (1982).

3. Lurie, I. S. and Wittwer, J.D., eds., High-Performance Liquid Chromatography in Forensic Chemistry, Marcel Dekker, New York, 1983, p. 161.

4. Willette, R.E. in Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, R.F. Doerge, ed., J.B. Lippincott, Philadelphia, 1982, p. 607.

5. White, M.W., J. Chromatogr., 178, 229 (1979).

6. Wallace, J.E., Harris, S.C., and Peek M.W., Anal. Chem., 52, 1328 (1980).

7. Vandenberghe, H., Macleod, S.M., Chinyanga, H. and Soldin, S.J., Thera. Drug Monitor., 4, 307 (1982).

8. Owen, J.A. and Sitar, D.S., J. Chromatogr., 276, 202 (1983).

9. Aitkenhead, A.R., Vater, M., Achola, K., Cooper, C.M. and Smith, G., Br. J. Anaesth., 56, 813 (1984).

10. Vater, M., Smith, G., Aherne, G.W. and Aitkenhead, A.R., Br. J. Anaesth., 56, 821 (1984).

11. Kotrly, K.J., Ebert, T.J., Vucins, E.J., Roerig, D. L. and Kampine, J.P., Anesthesiol., 61, 558 (1984).

12. Klippel, K., in Clinical Liquid Chromatography, Kabra, P.M. and Marton, L.J., eds., vol. 1, CRC Press, Boca Raton, 1984, p. 153.

13. Svensson, J., Rane, A., Säwe, J. and Sjöqvist, F., J. Chromatogr., 230, 427 (1982).

14. Todd, R.D., Muldoon, S.M. and Watson, R.L., J. Chromatogr., 232, 101 (1982).

15. Ishikawa, K., McGaugh, J.L., Shibanoki, S., and Kubo, T., Jap. J. Pharmacol., 32, 969 (1982).

16. Shibanoki, S. and Ishikawa, K., Jap. J. Pharmacol., 32, suppl. 254P (1982).

17. Kim, C. and Kats, T., J. Anal. Toxicol., 8, 135 (1984).

18. Olsen, G.D., Ousley, P.J. and Mathis, M.D., Neuropeptides, 5, 77 (1984).

19. Rane, A., Säwe, J., Lindberg, B., Svensson, J., Garle, M., Erwald, R. and Jorulf, H., J. Pharmacol. Exper. Therapeut., 229, 571 (1984).

20. Kemner, J.M., Snodgrass, W.R., Worley, S.E., Hodges, G.R., Clark, G.M. and Hignite, C.E., J. Lab. Clin. Med., 104, 433 (1984).

21. Tagliaro, F., Dorizzi, R., Plescia, M., Pradella, M., Ferrari, S., and Lo Cascio, V., Fresenius' Z. für Anal. Chem., 317, 678 (1984).

22. Ulrich, L. and Ruegsegger, P., Arch. Toxicol., 45, 241 (1980).

23. Visser, J., Grasmeijer, G. and Moolenaar, F., J. Chromatog., 274, 372 (1983).

24. Posey, B.L. and Kimble, S.N., J. Anal. Toxicol., 7, 241 (1983).

25. Posey, B.L. and Kimble, S.N., J. Anal. Toxicol., 8, 68 (1984).

26. Nitsche, V. and Mascher, H., J. Pharmaceut. Sci., 73, 1556 (1984).

27. Nelson, P.E., Fletcher, S.M. and Moffat, A.C., J. Forens. Sci. Soc., 20, 195 (1980).

28. Tsina, I.W., Fass, M., Debban, J.A., and Matin, S.B., Clin. Chem., 28, 1137 (1982).

29. Meinsma, D.A. and Kissinger, P.T., Current Separations, 6, 42 (1985).

30. Garrett, E.R. and Gürkan, T., J. Pharmaceut. Sci., 68, 26 (1979).

31. Umans, J.G., Chiu, T.S., Lipman, R.A., Schultz, M.F., Shin, S. and Inturrisi, C.E., J. Chromatogr., 233, 213 (1982).

32. Schneider, J.J., Triggs, E.J., Bourne, D.W., Stephens, I.D. and Haviland A.M., J. Chromatogr., 308, 359 (1984).

33. Barnes, J.N., Williams, A.J., Tomson, M.J., Toseland, P.A. and Goodwin, F.J., Br. Medical J., 290, 740 (1985).

34. Lake, C.L., DeFazio, C.A., Duckworth, E.N., Moscicki, J.C., Engle, J.S. and Durbin, C.G., J. Chromatogr., 233, 410 (1982).

35. Keegan, M. and Kay, B., J. Chromatogr., 311, 223 (1984).

36. Lo, M., Juergens, G.P., and Whitney, C.C., Res. Commun. Chem. Pathol. Pharmacol., 43, 159 (1984).

37. de Silva, J.A.F., Pao, J. and M.A. Brooks, in Drug Metabolite Isolation and Determination, E. Reid and J.P. Leppard, eds., Plenum, New York, 1983, p. 225.

38. Lucek, R. and Dixon, R., J. Chromatogr., 341, 239 (1985).

39. Gaver, R.C., Vasiljev, M., Wong, H., Monkovic, I., Swigor, J.E., Van Harken, D.R. and Smyth, R.D., Drug Metab. Disposit., 8, 230 (1980).

40. Anderson, R.D., Ilett, K.F., Dusci, L.J. and Hackett, L.P., J. Chromatogr., 227, 239 (1982).

41. Anderson, R.D. and Ilett, K.F., Clin. Exp. Pharmacol. Physiol., 9, 451 (1982).

42. Guay, D.R., Meatherall,R.C., Chalmers, J.L., and Grahame, G.R., Br. J. Clin. Pharmac., 18, 907 (1984).

43. Meatherall, R.C., Guay, D.R. and Chalmers, J.L., J. Chromatogr., 338, 141 (1985).

44. Derendorf, H. and E.R. Garrett, J. Pharmaceut. Sci., 72, 630 (1983).

45. Liu, S., Roerig, D.L. and Wang, R.I., Drug Metab. Disposit., 11, 335 (1983).

46. Roerig, D.L., Dawson, C.A. and Wang, R.I., Drug Metab. Disposit., 10, 230 (1982).

47. Kiang, C., Campos-Flor, S. and Inturrisi, C.E., J. Chromatogr., 222, 81 (1981).

48. Kunka, R.L., Yong, C., Ladik, C.F. and Bates, T.R., J. Pharmaceut. Sci., 74, 103 (1985).

49. Sutheimer, C., Hepler, B.R., Sebrosky, G.F. and Sunshine, I., Clin. Chem., 28, 1551 (1982).

50. Hepler, B.R., Sutheimer, C., Sunshine, I. and Sebrosky, G.F., J. Anal. Toxicol., 8, 78 (1984).

51. Vincent, P.G. and Engelke, B.F., J. Assoc. Off. Anal. Chem., 62, 310 (1979).

52. Hutin, M., Cave, A. and Foucher, J., J. Chromatogr., 268, 125 (1983).

53. Doner, L.W. and Hsu, A., J. Chromatogr., 253, 120 (1982).

54. Galewsky, S. and Nessler, C.L., Chromatographia, 18, 87 (1984).

55. Nobuhara, Y., Hirano, S., Namba, K. and Hashimoto, M., J. Chromatogr., 190, 251 (1980).

56. Hansen, S.H., Hansen, A.M. and Poulsen B., Arch. Pharm. Chemi. Sci. Ed., 8, 181 (1980).

57. Engelke, B.F. and Vincent, P.G., J. Assoc. Off. Anal. Chem., 65, 651 (1982).

58. Pettitt, B.C. and Damon, C.E., J. Chromatogr., 242, 189 (1982).

59. Aramaki, K., Hanai, T. and Walton, H.F., Anal. Chem., 52, 1965 (1980).

60. Hinds, C.J., Johnston, D. and Thompson, C.J., Drug Dev. Indust. Pharm., 10,983 (1984).

61. Harvey, H.E. and Chell, R.M., Austral. J. Pharm. Sci., 10, 115 (1981).

62. Jhangiani, R.K. and Bello, A.C., J. Assoc. Off. Anal. Chem., 68, 523 (1985).

63. Lee, M.G., J. Chromatogr., 312, 473 (1984).

64. Baker, D.E., Yost, G.S., Craig, V.L. and Campbell, R.K., Am. J. Hosp. Pharm., 42, 1352 (1985).

65. Majlat, P., Helboe, P. and Kristensen, A.K., Int. J. Pharmaceut., 9, 245 (1981).

66. Macias, J.M., Martin, W.J. and Lloyd, C.W., Am. J. Hosp. Pharm., 42, 1087 (1985).

67. Kubiak, E.J. and Munson, J.W., J. Pharmaceut. Sci., 69, 152 (1980).

68. Muhammad, N. and Bodnar, J.A., J. Liq. Chromatogr. 3, 113 (1980).

69. Ko, C.Y., Marziani, F.C. and Janicki, C.A., J. Pharmaceut. Sci., 69, 1081 (1980).

70. Gupta, V.D., J. Pharmaceut. Sci., 69, 110 (1980).

71. Stuber, V.B. and Müller, K.H., Pharmaceut. Acta Helv., 57, 181 (1981).

72. Poochikian, G.K. and Cradock, J.C., J. Chromatogr., 171, 371 (1979).

73. Poochikian, G.K. and Cradock, J.C., J. Pharmaceut. Sci., 69, 637 (1980).

74. Poochikian, G.K., Cradock, J.C. and Davignon, J.P., Int. J. Pharmaceut., 13, 219 (1983).

75. Beaumont, I.M.,Pharmaceut. J., 229,39 (1982).

76. Beaumont, I.M., Anal. Proc., 19, 128 (1982).

77. Wallo, W.E. and D'Adamo, A., J. Pharmaceut. Sci., 71, 1115 (1982).

78. Yang, S.L., Wilken, L.O. and Clark, C.R., Drug Dev. Indust. Pharm., 11, 799 (1985).

79. The U.S. Pharmacopeia. 21st ed., USP Convention, Inc., Rockville, Md., 1985, p. 767.

80. ibid, p. 142.

81. Kleinberg, M.L., Stauffer, G.L. and Latiolais, C.J., Am. J. Hosp. Pharm., 37, 680 (1980).

82. Wilson, T.D., J. Chromatogr., 243, 99 (1982).

83. Gupta, V.D., J. Pharmaceut. Sci., 72, 695 (1983).

84. Hsieh, J., Ma, J.K., O'Donnell, J.P. and Choulis, N.H., J. Chromatogr., 161, 366 (1978).

85. The U.S. Pharmacopeia. 21st ed., USP Convention, Inc., Rockville, Md., 1985, p. 649.

86. ibid, p. 650.

87. Levy, E. and Fecher, R., Clin. Chem., 28, 1551 (1982).

88. Barkan, S. and Wainer, I.W., J. Chromatogr., 240, 547 (1982).

89. Sa, S., Rashid, A. and Jalal, I., Talanta, 5, 397 (1984).

90. Baker, J.K., Skelton, R.E., Riley, T.N. and Bagley, J.R., J. Chromatogr. Sci., 18, 153 (1980).

91. Law, B., Gill, R. and Moffat, A.C., J. Chromatogr., 301, 165 (1984).

92. Lurie, I.S. and Demchuk, S.M., J. Liq. Chromatogr., 4, 357 (1981).

93. Noggle, F.T., J. Liq. Chromatogr., 6, 2005 (1983).

94. Baker, P.B. and Gough, T.A., J. Chromatogr. Sci., 19, 483 (1981).

95. Lurie, I.S., Sottolano, S.M. and Blasof,S., J. Forens. Sci., 27, 519 (1982).

96. Huizer, H., J. Forens. Sci., 28, 32 (1983).

97. Huizer, H., J. Forens. Sci., 28, 40 (1983).

98. Law, B., Joyce, J.R., Bal, T.S., Goddard, C.P., Japp, M. and Humphreys, I.J., Anal. Proc., 20, 611 (1983).

99. White, P.C., Jane, I., Scott, A., and Connett, B.E., J. Chromatogr., 265, 293 (1983).

100. Laurent, C.J., Billiet, H.A. and De Galan, L., J. Chromatogr., 285, 161 (1984).

101. Lurie, I.S. and Allen, A.C., J. Chromatogr., 317, 427 (1984).

102. Baker, J.K., Skelton, R.E. and Ma, C., J. Chromatogr., 168, 417 (1979).

103. Fell, A.F., Scott, H.P., Gill, R. and Moffat, A.C., J. Chromatogr., 273, 3 (1983).

104. Nelson, P.E., Nolan, S.L. and Bedford, K.R., J. Chromatogr., 234, 407 (1982).

105. Nelson, P.E., J. Chromatogr., 298, 59 (1984).

106. Peterson, R.G., Rumack, B.H., Sullivan, J.B. and Makowski, A., J. Chromatogr., 188, 420 (1980).

107. Wilson, T.D., J. Chromatogr., 301, 39 (1984).

108. Wilson, T.D., J. Chromatogr., 298, 131 (1984).

109. Jane, I., McKinnon, A. and Flanagan, R.J., J. Chromatogr., 323, 191 (1985).

110. Hansen, S.H., J. Chromatogr., 209, 203 (1981).

111. Hansen, S.H., J. Chromatogr., 212, 229 (1981).

112. Lindberg, W., Johansson, E. and Johansson, K., J. Chromatogr., 211, 201 (1981).

113. Proksa, B., Pharmazie, 39, 687 (1984).

114. Popl, M., Ky, L.D. and Strnadová, J., J. Chromatogr. Sci., 23, 95 (1985).

115. Lurie, I.S., Allen, A.C. and Issaq, H.J., J. Liq. Chromatogr., 7, 463 (1984).

116. Lurie, I.S. and Allen, A.C., J. Chromatogr., 292, 283 (1984).

117. Blair, D., Rumack, B.H. and Peterson, R.G., Clin. Chem., 24, 1543 (1978).

118. Terweij-Groen, C.P., Vahlkamp, T. and Kraak, J.C., J. Chromatogr., 145, 115 (1978).

119. Liversidge, G.G., Nishihata, T., Higuchi, T., Shaffer, R. and Cortese, M., J. Chromatogr., 276, 375 (1983).

120. Borgström, L., Ekman, B., Larsson, H., Leden, I., Lindahl, A., Melander, A. and Wåhlin-Boll, E., Biopharm. and Drug Disposit., 5, 261 (1984).

121. Cham, B.E., Johns, D., Bochner, F., Imhoff, D.M. and Rowland, M., Clin. Chem., 25, 1420 (1979).

122. Cham, B.E., Bochner, F., Imhoff, D.M., Johns, D. and Rowland, M., Clin. Chem., 26, 111 (1980).

123. Maulding, D.L. and Young, J.F., J. Pharmaceut. Sci., 69, 1224 (1980).

124. Edgar, B., Bogentoft, C. and Lagerström, P., Biopharm. and Drug Disposit., 5, 251 (1984).

125. Clark, J.H., Nagamori, K. and Fitzgerald, J.F., Clin. Chim. Acta, 145, 243 (1985).

126. Peng, G.W., Gadalla, M.A., Smith, V., Peng, A. and Chiou, W.L., J. Pharmaceut. Sci., 67, 710 (1978).

127. Harrison, L.I., Funk, M.L. and Ober, R.E., J. Pharmaceut. Sci., 69,1268 (1980).

128. Cham, B.E., Ross-Lee, L., Bochner, F. and Imhoff, D.M., Therap. Drug Monitor., 2, 365 (1980).

129. Lo, L.Y. and Bye, A., J. Chromatogr., 181, 473 (1980).

130. Rumble, R.H., Roberts, M.S. and Wanwimolruk, S., J. Chromatogr., 225, 252 (1981).

131. Buskin, J.N., Upton, R.A. and Williams, R.L., Clin. Chem., 28, 1200 (1982).

132. Bakar, S.K. and Niazi, S., J. Pharmaceut. Sci., 72, 1020 (1983).

133. Reidl, U., J. Chromatogr., 272, 325 (1983).

134. Nieder, M. and Jaeger, H., J. High Resoln. Chromatogr. Chromatogr. Comm., 6, 655 (1983).

135. O'Kruk, R.J., Adams, M.A. and Philp, R.B., J. Chromatogr., 310, 343 (1984).

136. Mays, D.C., Sharp, D.E., Beach, C.A., Kershaw, R.A., Bianchine, J.R. and Gerber, N., J. Chromatogr., 311, 301 (1984).

137. Brandon, R.A., Eadie, M.J. and Smith, M.T., Therapeut. Drug Monitor., 7, 216 (1985).

138. Miceli, J.N., Aravind, M.K., Cohen, S.N. and Done, A.K., Clin. Chem., 25, 1002 (1979).

139. Wilson, J.T., Therapeut. Drug Monitor., 1, 459 (1979).

140. Ou, C. and Frawley, V.L., Clin. Chem., 28, 2157 (1982).

141. Ferrell, W.J. and Goyette, G.W., J. Liq. Chromatogr., 5, 93 (1982).

142. Meinsma, D.A., Radzik, D.M. and Kissinger, P.T., J. Liq. Chromatogr., 6, 2311 (1983).

143. DeLuccia, F.J., Arunyanart, M. and Love, L.J., Anal. Chem., 57, 1564 (1985).

144. Morris, M.E. and Levy, G., J. Pharmaceut. Sci., 72, 612 (1983).

145. Fielding, R.M., Waschek, J.A., Rubin, G.M., Pond, S.M. and Tozer, T.N., J. Liq. Chromatogr., 7, 1221 (1984).

146. Gupta, V.D., J. Pharmaceut. Sci., 69, 113 (1980).

147. Kirchhoefer, R.D., J. Pharmaceut. Sci., 69, 1188 (1980).

148. Taguchi, V.Y., Cotton, M.L., Yates, C.H. and Millar, J.F., J. Pharmaceut. Sci., 70, 64 (1981).

149. Galante, R.N., Wisalli, A.J. and Grim, W.M., J. Pharmaceut. Sci., 73, 195 (1984).

150. Bevitt, R.N., Mather, J.R. and Sharman, D.C., Analyst, 109, 1327 (1984).

151. Fogel, J., Epstein, P. and Chen, P., J. Chromatogr., 317, 507 (1984).

152. The U.S. Pharmacopeia. 21st ed., USP Convention, Inc., Rockville, Md., 1985, p. 78.

153. Ali, S.L., J. Chromatogr., 126, 651 (1976).

154. Reepmeyer, J.C. and Kirchhoefer, R.D., J. Pharmaceut. Sci., 68, 1167 (1979).

155. Kirchhoefer, R.D. and Juhl, W.E., J. Pharmaceut. Sci., 69, 548 (1980).

156. Kirchhoefer, R.D., Reepmeyer, J.C. and Juhl, W.E., J. Pharmaceut. Sci., 69, 550 (1980).

157. Pfeiffer, C.D. and Pankey, J.W., J. Pharmaceut. Sci., 71, 511 (1982).

158. Matsuda, R., Yamamiya, T., Tatsuzawa, M., Emima, A. and Takai, N., J. Chromatogr., 173, 75(1979).

159. Honigberg, I.L., Stewart, J.T. and Smith, M., J. Pharmaceut. Sci., 67, 675 (1978).

160. Stewart, J.T., Honigberg, I.L. and Coldren, J.W., J. Pharmaceut. Sci., 68, 32 (1979).

161. Haddad, P., Hutchins, S. and Tuffy, M., J. Chem. Ed., 60, 166 (1983).

162. Williams, K.J., Po, A.L. and Irwin, W.J., J. Chromatogr., 194, 217 (1980).

163. Gfeller, J.C., Haas, R., Troendle, J.M. and Erni, R., J. Chromatogr., 294, 247 (1984).

164. Gupta, V.D., J. Pharmaceut. Sci., 69, 110 (1980).

165. Kagel, R.A. and Farwell, S.O., J. Chem. Ed., 60, 163 (1983).

166. Beaver, R.W., Bunch, J.E. and Jones, L.A., J. Chem. Ed., 60, 1000 (1983).

167. Krieger, D.J., J. Assoc. Off. Anal. Chem., 67, 339 (1984).

168. Fletterick, C.G., Grove, T.H. and Hohnadel, D.C., Clin. Chem., 25, 409 (1979).

169. Quattrone, A.J. and Putnam, R.S., Clin. Chem., 27, 129 (1981).

170. West, J.C., J. Anal. Toxicol., 5, 118 (1981).

171. Wal, S., Bannister, S.J. and Snyder, L.R., J. Chromatogr. Sci., 20, 260 (1982).

172. Miner, D.J. and Kissinger, P.T., J. Pharmaceut. Sci., 68, 96 (1979).

173. Walsh, F.X., Langlais, P.J. and Bird, E.D., Clin. Chem., 28, 382 (1982).

174. Klippel, K., in Clinical Liquid Chromatography, Kabra, P.M. and Marton, L.J., eds., vol. 1, CRC Press, Boca Raton, 1984, p.5.

175. Knox, J.H. and Jurand, J., J. Chromatogr., 149, 297 (1978).

176. Miners, J., Adams, J.F. and Birkett, D.J., Clin. Experim. Pharmacol. Physiol., 11, 209 (1984).

177. Hart, S.J., Aguilar, M.I., Healey, K., Smail, M.C. and Calder, I.C., J. Chromatogr., 306, 215 (1984).

178. Hamilton, M. and Kissinger, P.T., Anal. Biochem., 125, 143 (1982).

179. Wilson, J.M., Slattery, J.T., Forte, A.J. and Nelson, S.D., J. Chromatogr., 227, 453 (1982).

180. Munson, J.W., Weierstall, R. and Kostenbauder, H.B., J. Chromatogr., 145, 328 (1978).

181. Lo, L.Y. and Bye, A., J. Chromatogr., 173, 198 (1979).

182. Ameer, B., Greenblatt, D.J., Divoll, M., Abernethy, D.R. and Shargel, L., J. Chromatogr., 226, 224 (1981).

183. Korduba, C.A. and Petruzzi, R.F., J. Pharmaceut. Sci., 73, 117 (1984).

184. Fritz, A.K., Benziger, D.P., Peterson, J.E., Park, G.B. and Edelson, J., J. Pharmaceut. Sci., 73, 326 (1984).

185. Pang, K.S., Taburet, A.M., Hinson, J.A. and Gillette, J.R., J. Chromatogr., 174, 165 (1979).

186. Miner, D.J. and Kissinger, P.T., Biochem. Pharmacol., 28, 3285 (1979).

187. Gemborys, M.W. and Mudge, G.H., Drug Metab. Disposit., 9, 340 (1981).

188. Hart, S.J., Tontodonati, R. and Calder, I.C., J. Chromatogr., 225, 387 (1981).

189. Jung, D. and Zafar, N.U., J. Chromatogr., 339, 198 (1985).

190. Moldéus, P., Biochem. Pharmacol., 27, 2859 (1978).

191. Morgan, M.E. and Freed, C.R., J. Pharmacol. Experim. Therap., 219, 49 (1981).

192. Fayz, S., Cherry, W.F., Dawson, J.R., Mulder, G.J. and Pang, K.S., Drug Metab. Disposit., 12, 323 (1984).

193. Watari, N., Iwai, M. and Kaneniwa, N., J. Pharmacokin. Biopharm., 11, 245 (1983).

194. Jung, D., J. Pharmacol. Experim. Therap., 232, 178 (1985).

195. Munson, J.W. and Kubiak, E.J., Anal. Lett., 13, 705 (1980).

196. Sena, F.J., Piechocki, J.T. and Li, K.L., J. Pharmaceut. Sci., 68, 1465 (1979).

197. Gilpin, R.K. and Gaudet, M.H., J. Chromatogr., 248, 160 (1982).

198. Quaglia, M.G., Mazzeo, P. and Lopez, A., J. Pharmaceut. Biomed. Anal., 3, 187 (1985).

199. Carnevale, L., J. Pharmaceut. Sci., 72, 196 (1983).

200. The U.S. Pharmacopeia. 21st ed., USP Convention, Inc., Rockville, Md., 1985, p. 14.

201. Mamolo, M.G., Vio, L. and Maurich, V., J. Pharmaceut. Biomed. Anal., 3, 157 (1985).

202. Gupta, V.D. and Heble, A.R., J. Pharmaceut. Sci., 73, 1553 (1984).

203. Thomis, R., Roets, E. and Hoogmartens, J., J. Pharmaceut. Sci., 73, 1830 (1984).

204. Baker, J.K. and Ma, C., J. Chromatogr., 169, 107 (1979).

205. Miner, D.J., Anal. Chim. Acta, 134, 101 (1982).

206. Hoshino, T., Senda, M., Hondo, T., Saito, M. and Tohei, S., J. Chromatogr., 316, 473 (1984).

207. Kearns, G.L. and Wilson, J.T., J. Chromatogr., 226, 183 (1981).

208. Shimek, J.L., Rao, N.G. and Khalil, S.K., J. Pharmaceut. Sci., 70, 514 (1981).

209. Aravind, M.K., Miceli, J.N. and Kauffman, R.E., J. Chromatogr., 308, 350 (1984).

210. Lockwood, G.F. and Wagner, J.G., J. Chromatogr., 232, 335 (1982).

211. Albert, K.S., Raabe, A., Garry, M., Antal, E.J. and Gillespie, W.R., J. Pharmaceut. Sci., 73, 1487 (1984).

212. Greenblatt, D.J., Arendt, R.M. and Locniskar, A., Arzneim.-Forsch., 33, 1671 (1983).

213. Litowitz, H., Olanoff, L. and Hoppel, C.L., J. Chromatogr., 311, 443 (1984).

214. Conrad, K.A., Mayersohn, M. and Bliss, M., Br. J. Clin. Pharmac., 18, 624 (1984).

215. Ali, A., Kazmi, S. and Plakogiannis, F.M., J. Pharmaceut. Sci., 70, 944 (1981).

216. Askholt,J. and Nielsen-Kudsk, F., Acta Pharmacol. Toxicol., 56, 99 (1985).

217. Maitre, J., Boss, G. and Testa, B., J. Chromatogr., 299, 397 (1984).

218. Lee, E.J., Williams, K.M., Graham, G.G., Day, R.O. and Champion, G.D., J. Pharmaceut. Sci., 73, 1542 1984.

219. Wainer, I.W. and Doyle, T.D., J. Chromatogr., 284, 117 (1984).

220. Wainer, I.W. and Doyle, T.D., Liq. Chromatogr., 2, 88 (1984).

221. Dusci, L.J. and Hackett, L.P., J. Chromatogr., 172, 516 (1979).

222. Levine, B. and Caplan, Y.H., Clin. Chem., 31, 346 (1985).

223. Tsao, J.C. and Savage, T.S., Drug Dev. Indust. Pharm., 11, 1123 (1985).

224. Upton, R.A., Buskin, J.N., Guentert, T.W., Williams, R.L. and Riegelman, S., J. Chromatogr., 190, 119 (1980).

225. Broquaire, M., Rovei, V. and Braithwaite, R., J. Chromatogr., 224, 43 (1981).

226. Burgoyne, R.F., Brown, P.R. and Kaplan, S.R., J. Liq. Chromatogr., 3, 101 (1980).

227. Høylandskjaer, A. and Aarbakke, J., Acta Pharmacol. Toxicol., 52, 78 (1983).

228. Wahlund, K.G. and Arvidsson, T., J. Chromatogr., 282, 527 (1983).

229. Ng, K. and Snyderman, T., J. Chromatogr., 178, 241 (1979).

230. Grindel, J.M., Hills, J.F. and Renzi, N.L., J. Chromatogr., 272, 210 (1983).

231. Welch, C.L., Annesley, T.M., Luthra, H.S. and Moyer, T.P., Clin. Chem., 28, 481 (1982).

232. Desiraju. R.K., Nayak, R.K. and Pritchard, J.F., J. Clin. Pharmacol., 24, 371 (1984).

233. Langendijk, P.N., Smith, P.C., Hasegawa, J. and Benet, L.Z., J. Chromatogr., 307, 371 (1984).

234. Müller, H. and Zulliger, H.W., Arzneim.-Forsch., 35, 152 (1985).

235. Aarons, L. and Higham, C., Clin. Chim. Acta, 105, 377 (1980).

236. Spahn, V.H. and Mutschler, E., Arzneim.-Forsch., 31, 495 (1981).

237. Sioufi, A., Colussi, D. and Mangoni, P., J. Chromatogr., 275, 201 (1983).

238. Weyhenmeyer, V.R., Dingler, E., Lohaus, R., Mayer, D., Teicher, H., Wybitul, K., Wangermann, G. and Steim, H., Arzneim.-Forsch., 34, 721 (1984).

239. Alvinerie, M., J. Chromatogr., 181, 132 (1980).

240. Martin, K., Stridsberg, M.I. and Wiese, B.M., J. Chromatogr., 276, 224 (1983).

241. Marunaka, T., Shibata, T., Minami, Y. and Umeno, Y., J. Chromatogr., 183, 331 (1980).

242. Tsai, Y. and Naito, S., Int. J. Pharmaceut., 23, 315 (1985).

243. De Vries, J.X., Staiger, C., Wang, N.S., Schlicht, F. and Koroknay, B., J. Chromatogr., 309, 219 (1984).

244. Teunissen, M.W., Meerburg, J.E., Vermeulen, N. P. and Breimer, D.D., J. Chromatogr., 278, 367 (1983).

245. Weber, A., Opheim, K. and Smith, A.L., J. Chromatogr. Sci., 22, 239 (1984).

246. Kahn, G.C., Boobis, A.R., Blair, I.A., Brodie, M.J. and Davies, D.S., Anal. Biochem., 113, 292 (1981).

247. Danhof, M., Vis, E. and Breimer, D.D., Pharmacol., 18, 210 (1979).

248. Campbell, T.M., Murdaugh, E.W. and Killenberg, P.G., J. Chromatogr., 163, 236 (1979).

249. Nakagawa, A., Nakamura, K., Ishizaki, T. and Chiba, K., J. Chromatogr., 231, 349 (1982).

250. Shargel, L., Cheung, W. and Yu, A.B., J. Pharmaceut. Sci., 68, 1052 (1979).

251. Rhodes, J.C. and Houston, J.B., Biopharm. Drug Disposit., 4, 125 (1983).

252. Griffeth, L.K., Rosen G.M., Tschanz, C. and Rauckman, E.J., Drug Metab. Disposit., 11, 517 (1983).

253. De Beer, J.O., Jacobs, G.A. and Martens, M.A., J. Chromatogr., 307, 475 (1984).

254. Guinebault, P. and Broquaire, M., J. Chromatogr., 217, 509 (1981).