except for dilutions. This procedure is much more convenient than extracting the steroid with a solvent that must be removed before determination. The insoluble constituents in these tablets do not cause any interference and need not be removed before differential pulse polarography. The drug is quantitatively dissolved in the solvent upon shaking for 20 min.

This differential pulse polarographic procedure offers the advantages of sensitivity, speed of analysis, and moderate cost of apparatus. Since the samples are analyzed without prior separation, the procedure results in a considerable saving of time, particularly when a large number of samples are assayed or when a large number of single tablets must be assayed to establish content uniformity.

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ACKNOWLEDGMENTS

Adapted in part from a thesis submitted by R. N. Yadav to the University of Toronto in partial fulfillment of the Master of Science degree requirements.

The authors are indebted to the pharmaceutical companies for their generous contributions of reference standards and dosage forms.

COMMUNICATIONS

Assay of Amygdalin Dosage Forms from Mexico

Keyphrases □ Amygdalin dosage forms—IR and NMR spectral identification and high-pressure liquid chromatographic analysis of components in various products □ IR spectroscopy—identification, components in various amygdalin dosage forms □ NMR spectroscopy—identification, components in various amygdalin dosage forms □ High-pressure liquid chromatography—analyses, amygdalin and components in various dosage forms

To the Editor:

One controversial topic in cancer treatment today is whether laetrile (1) (amygdalin[1]) is effective as a cancer chemotherapeutic agent. To resolve this complicated issue, the National Cancer Institute (NCI) considered a clinical trial to test the efficacy of amygdalin in cancer treatment. "Available" amygdalin dosage forms were procured and evaluated to determine their suitability for such a trial. Although the question of efficacy in humans may not be answered for a long time, the chemical compositions of the title amygdalin formulations are now known. Because this information has not been previously available to the public[2], it should be of vital interest to current and future users of these dosage forms. For this reason, we are reporting our findings in this communication.

Most amygdalin formulations used by cancer patients in this country are produced in Mexico, and samplings of the products we assayed originated from that country. Our samplings were selected randomly from groupings of these formulations released to NCI by U.S. Customs, which had seized the amygdalin products as they were being transported illegally into the United States. The seized materials consisted of five groups[3] of injectable liquids in amber ampuls and three groups[3] of tablets for oral administration. The amber ampuls had a 10-ml fill volume capacity. A ceramic label indicated the contents as "Amigdalina 3 g." and identified the supplier as Cyto Pharma de Mexico, S.A. The label also stated that the product was an injec-

[1] The trivial names amygdalin, laetrile, nitriloside, vitamin B-17, and 1-mandelonitrile-β-diglycoside have been used synonymously by both the proponents and opponents of laetrile (2). Systematically, laetrile is (*R*)-mandelonitrile-β-glucuronic acid (I) and amygdalin is (*R*)-mandelonitrile-β-D-glucosido-6-β-D-glucoside (II). The drug currently offered as an antitumor agent is amygdalin, which is usually extracted from kernels of apricots and related fruits.

[2] Levi *et al.* (3) described briefly the physicochemical and biochemical properties of two pharmaceutical formulations of amygdalin available in the United States and Canada in the early 1960's. The authors identified neither the manufacturers nor the dosage potencies. However, a comparison of the reported data with those in this article indicates that the earlier products differed from those available today.

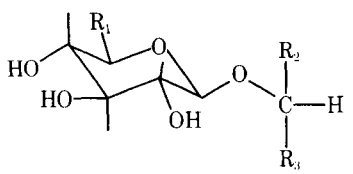
[3] According to U.S. Customs records, these groups were seized between 1973 and 1975; all groups, except Group 76-2505-11569, contained at least 1000 dosage units. The seized materials were kept in an air-conditioned vault from the time of seizure to the time of release, July 1977.

Table I—Assay Results for the Five Groups of Injectables

U.S. Customs Group (Seizure Date)	Ampul	(*R,S*)-Amygdalin, g/Ampul	Percent Label, 3 g/Ampul
74-2504-10919 (12/28/1973)	1	1.64	54.7
	2	1.64	54.7
	3	1.64	54.7
	4	1.53	51.0
	5	1.65	55.0
Average		1.62	54.0
SD		0.05	1.7
75-2504-10716 (11/09/1974)	1	1.65	55.0
	2	1.68	56.0
	3	1.68	56.0
	4	1.64	54.7
	5	1.65	55.0
Average		1.66	55.3
SD		0.02	0.6
75-2504-11744 (05/21/1975)	1	1.40	46.7
	2	1.39	46.3
	3	1.38	46.0
	4	1.37	45.7
	5	1.38	46.0
Average		1.38	46.1
SD		0.01	0.4
76-2505-11569 (04/11/1975)	1	1.18	39.3
	2	1.19	39.7
	3	1.72	57.3
	4	1.68	56.0
Average		1.44	48.1
SD		0.30	9.9
76-2810-00001 (12/12/1975)	1	1.39	46.3
	2	1.45	48.3
	3	1.42	47.3
	4	1.52	50.7
Average		1.44	48.2
SD		0.06	1.9

table solution. The tablets were uncoated, round, and yellow; they measured approximately 12 × 3 mm. On one side was inscribed "500"; on the other was an outline of a conifer, a logo used by Cyto Pharma de Mexico, S. A. Manufacturing control numbers, expiration dates, and storage recommendations were not apparent on product labeling or packaging.

Injectables—Identification of the "active" component(s) in the injectables was based on spectral and chromatographic evidence. IR data were obtained from the freeze-dried residues of the solutions. IR spectra from all five lots were very similar, showing bands that were rather broad. Absorptions in the 2.8–3.3- and 9–10-μm regions were consistent with those expected for carbohydrates. Bands at 13.2 and 14.3 μm were in agreement with a phenyl; those at ~6.0 and 6.25 μm were suggestive of a primary amide. A very weak band at 4.4 μm was indicative of a nitrile. The proton NMR spectra for all five lots, obtained from the solutions, were comparable and consistent with the structure of amygdalin. However, in the δ 5–6-ppm region, where the benzylic proton was expected, four singlets appeared at δ 5.1, 5.4, 5.9, and 6.2 ppm; in the δ 1.10-ppm region, where no signal was expected, a doublet



I (laetrile): R_1 = COOH, R_2 = CN, R_3 = C_6H_5

II [(*R*)-amygdalin]: R_1 = CH_2O-β-D-glucoside, R_2 = CN, R_3 = C_6H_5

III [(*S*)-amygdalin]: R_1 = CH_2O-β-D-glucoside, R_2 = C_6H_5, R_3 = CN

appeared. The δ 6.2- and 5.9-ppm resonances were identified as the benzylic proton in (*S*)- and (*R*)-amygdalins, respectively; the δ 5.1- and 5.4-ppm peaks were assigned the benzylic proton in amygdalinamide[4] and amygdalin acid[4], respectively; and the δ 1.10-ppm doublet was assigned to the methyls in 2-propanol[5]. The ratios of (*R*)- to (*S*)-amygdalins in all five batches were 1:1, within experimental error.

GLC–mass spectral analyses of the trimethylsilyl and trifluoroacetyl[6] derivatives of these formulations on methyl silicone (OV-1) and a trichlorophenyl methyl silicone (Versilube F-50)[6] columns, respectively, showed that the (*R*)- and (*S*)-amygdalins were separated; they yielded identical mass spectra. The GLC–mass spectral data also showed amygdalinamide plus another impurity, tentatively identified as 1-*O*-benzylgentiobiose. No evidence of laetrile [(*R*)-mandelonitrile-β-glucuronic acid] was found.

High-pressure liquid chromatographic (HPLC) analysis on a C_8-bonded silica (Lichrosorb RP8) column with aqueous methanol[7] readily separated amygdalin[7], amygdalinamide[7], and amygdalin acid[7]. Aliquots of 1.00 ml from ampuls of each group were each transferred to a 50-ml volumetric flask. After filling to mark with water, 1.00-ml aliquots of the resulting solutions were each mixed with 0.50 ml of an internal standard solution[8] before injection. The concentrations of amygdalin per milliliter were determined by the internal standard method; the amygdalin reference was Pharm-Eco lot PC-0237A[9]. The amount of (*R,S*)-amygdalin per ampul was obtained by multiplying the ampul solution volume[10] times its solution concentration. The results are shown in Table I.

Since these ampuls were not identified by manufacturing control numbers, it is possible that a single seizure group number could contain more than one production batch. The results for Group 76-2505-11569 suggest this possibility.

Tablets—Identification of the "active" component in the tablets was based on spectral and chromatographic evidence. Tablets from each lot were separately ground and extracted with methanol. Then the methanol solutions were evaporated to dryness, and the residues were used for spectral analysis. Recorded as mineral oil[11] mulls, the IR spectra from all three lots were comparable; they were also comparable to those obtained from the injectables. Re-

[4] Amygdalinamide and amygdalin acid are hydrolysis products of amygdalin in which the nitrile is hydrolyzed. They were the major contaminants in all five groups of ampuls. The (*R,S*)-epimers of each have been prepared in our laboratory; estimations of these contaminants in the subject injectables will appear in another report.

[5] 2-Propanol was a significant contaminant in these formulations; its quantitations will appear in another report.

[6] The use of this derivative and column was suggested by Dr. T. Cairns of the Food and Drug Administration.

[7] By varying the methanol–water ratio, epimeric pairs of the respective compounds could be separated. However, the time required to separate the (*R*)- and (*S*)-amygdalins was in excess of 1 hr; for this reason, the HPLC assay conditions were adjusted so that (*R*)- and (*S*)-amygdalins eluted as a single component.

[8] Prepared by dissolving 5 mg of *m*-nitroaniline (EKC) in water in a 50-ml volumetric flask.

[9] This reference was analyzed for 93.0 ± 1.5% (*R,S*)-amygdalin, 2–3% UV-absorbing impurities including ≥0.7% 1-*O*-benzylgentiobiose and ≥0.3% amygdalinamide, and 3.5 ± 0.3% water; the remainder of the sample was not identified. The equivalents of the UV characteristics for (*R*)- and (*S*)-amygdalins have been established.

[10] Each ampul fill volume was determined by carefully scoring the liquid level in each ampul, emptying and drying the ampul, and refilling to the scored mark with water dispensed from a volumetric buret. The average of six ampul volumes was 10.0 ± 0.1 ml.

[11] Nujol.

Table II—Assay Results for the Three Tablet Dosage Forms

U.S. Customs Group 76-2505-11569, Seized 4/11/75			U.S. Customs Group 75-2504-11744, Seized 5/21/75			U.S. Customs Group 75-2501-00107, Seized 6/24/76		
Tablet Weight, mg	Amygdalin per Tablet, mg	% Label[a]	Tablet Weight, mg	Amygdalin per Tablet, mg	% Label[a]	Tablet Weight, mg	Amygdalin per Tablet, mg	% Label[a]
555.0	285	57.0	543.8	423	84.6	548.5	436	87.1
539.1	265	53.0	544.6	457	91.5	554.3	420	84.1
537.6	278	55.6	545.7	419	83.8	553.1	413	82.7
540.5	279	55.8	540.6	439	87.8	545.1	428	85.7
537.2	274	54.9	540.1	428	85.6	554.8	419	83.7
542.3	274	54.9	542.7	453	90.5	553.9	446	89.3
Average 541.1	275.7	55.2	542.9	436.5	87.3	551.6	427.2	85.4
SD 4.7	6.6	1.3	2.2	15.9	3.2	3.9	12.3	2.5

[a] Based on the label amount as 500 mg/tablet.

Table III—Tablet Weights (in Milligrams)

Group	High	Low	Average
76-2505-11569	566.3	525.4	541.5, *SD* = 7.6
75-2504-11744	548.4	528.0	541.9, *SD* = 4.4
75-2501-00107	564.4	545.8	553.4, *SD* = 4.7

corded as solutions in deuterated dimethyl sulfoxide, the proton NMR spectra for all three lots were qualitatively identical to a spectrum of authentic (*R*)-amygdalin[12]. These spectra showed only one singlet, δ 5.9 ppm, in the δ 5–6-ppm region, indicating that the amygdalin in these tablets was the epimerically pure (*R*)-form. The lack of a signal in the δ 5.1–5.4-ppm region indicated the absence of amygdalinamide and amygdalin acid. This indication was verified by HPLC data that showed very minor amounts (~1.0% total[13]) of these two contaminants. Ground tablets were also extracted with deuterium oxide. Proton NMR spectra of these solutions showed little or no 2-propanol in these tablets.

These tablets were assayed by the HPLC method used for the injectables. Six tablets from each group were individually crushed, and each was transferred to a 100-ml volumetric flask with the aid of water. The mixtures were briefly sonicated and then thoroughly agitated. The yellow mixtures were brought to mark with more water and then filtered through 0.45-μm filters[14]. Each 1.00-ml aliquot of the clear yellow filtrates was mixed with 0.50 ml of an internal standard solution[8] before it was injected. Quantifications were based on the internal standard method; the amygdalin reference employed was Pharm-Eco lot PC-0237A III[9]. The results are given in Table II.

In addition, 20 tablets from each group were randomly selected and individually weighed. The results are listed in Table III.

Through this study, analytical methods were developed to assay injectable and oral dosage forms of amygdalin produced in Mexico. The results indicate that all of the dosage forms analyzed were subpotent. The average results for the five groups of injectables ranged from 46 to 55% of label; the three groups of tablets ranged from 55 to 87% of label. In addition, the amygdalin in the injectables was a mixture of (*R*)- and (*S*)-amygdalins; the amygdalin in the tablets was the pure (*R*)-form. The injectable dosage forms were also heavily contaminated by amygdalinamide, amygdalin acid, and 2-propanol.

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Received October 28, 1977.
Accepted for publication January 4, 1978.
Supported by Contract N01-CM-33723 from the National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.
The authors thank E. Shingai and D. Thomas of SRI International for assistance in GLC and mass spectral determinations and J. P. Davignon of NCI for valuable comments and suggestions.

[12] Lot 3634, Aldrich Chemical Co., analyzed for 95 ± 1.5% (*R*)-amygdalin, 1% 1-*O*-benzylgentiobiose, ≤0.4% amygdalinamide and other organic impurities, and 3 ± 0.3% water.
[13] Similar UV spectral characteristics at 254 nm for amygdalin, amygdalinamide, and amygdalin acid were assumed.
[14] Millipore.