1985). Examination of the compositional data shows that for *Amarulum* and *Amarum* the most preferred selections have higher percentages of lavandulol. Further animal studies are needed and will be conducted to establish the significance of these data.

Establishing a relationship between a given volatile or group of volatiles and grazing preference is difficult. Environmental factors (Arnold and Hill, 1972), as well as time and number of cuttings (Scehovic et al., 1985), have been shown to have an effect on the chemical composition of forage. While some trends appear, investigations are under way to establish possible correlation between the volatile profile and grazing preference in cattle using more closely controlled animal studies.

Registry No. $HO(CH_2)_5Me$, 111-27-3; *cis*-$HO(CH_2)_2CH{=}CHEt$, 928-96-1; *trans*-$HOCH_2CH{=}CHPr$, 928-95-0; $CH_2{=}CHCH(OH)CH_2Bu$, 3391-86-4; $MeCH(OH)(CH_2)_3Bu$, 628-99-9; $MeCH(OH)(CH_2)_2CH{=}CMe_2$, 1569-60-4; linalool, 78-70-6; lavandulol, 498-16-8; borneol, 507-70-0.

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Tocopherols of Soybean Seeds and Soybean Curd (Tofu)

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α-, γ-, and δ-tocopherols from soybean seeds and soybean curd (tofu) were extracted, separated, and quantitated by high-performance bonded normal-phase liquid chromatography with ultraviolet (UV) detection at 280 nm. Two successive extractions with ethanol at a solvent to dry matter ratio of about 20:1 were performed. Saponification was not necessary. The tocopherols were separated in 18 min in an amino column by using 1% isopropyl alcohol in hexane as the mobile phase. Tocopherol content varied significantly from one soybean variety to another. The amounts of α-, γ-, and δ-tocopherols in the soybeans ranged from 10.9 to 28.4, 150 to 191, and 24.6 to 72.5 μg/g (dry basis), respectively. Processing of soybeans into tofu results in a loss of vitamin E (47% and 30% for two varieties analyzed), but the tofu is a greater source of tocopherols than the soybeans on a dry basis. Storage of the curds for 15 days under commercial conditions does not affect vitamin E content.

The good nutritional properties of soybean food products rest largely on the high content and quality of the soybean protein. A number of nitrogen balance studies have suggested that the digestibility and biological value of soybean protein for humans are satisfactory and compare favorably with animal proteins (Liener, 1972). Tofu, a highly hydrated cheeselike product made by coagulation of the protein present in a soybean extract or soy milk, is a regular item in American supermarkets now. A high proportion of polyunsaturated lipids and lack of cholesterol are additional nutritional characteristics used in the marketing of tofu.

The level of vitamins and minerals in tofu is not well documented in the literature even though it is occasionally referred to in the popular literature. Miller et al. (1952) studied the retention of calcium, iron, thiamin, riboflavin, and niacin in commercially prepared soybean curd. Values for the content of some vitamins and minerals in soybeans and tofu are also listed in USDA Handbooks No. 8 (Agricultural Research Service, 1968) and No. 456 (Agricultural Research Service, 1975). These values are the averages of two samples that are made from unknown soybean varieties, by unknown processing methods, and for which older analytical methodology is employed. One of the vitamins not listed in the handbooks is vitamin E. Vitamin E activity in foods derives from two distinct series of compounds, the tocopherols and the tocotrienols. Many values for vitamin E levels in foods have been based in total tocopherol determinations based upon the Emmerie and Engel reaction (Ames, 1967). Practically, total tocopherol values thus obtained are a measure of the total reducing materials remaining after a series of purification procedures. Separation and quantitation of the individual tocopherols and tocotrienols became more common with the refinement of chromatographic techniques.

α-Tocopherol has the highest biological activity. In the past, it was the only compound considered in dietary calculations (National Research Council, 1980). Changing dietary fat patterns in the United States have resulted in soybean oil becoming the predominant dietary fat, and, consequently, average diets may have twice as much γ-tocopherol as α-tocopherol (Bieri and Evarts, 1974). In general, each one of the vitamin compounds has different vitamin E activities and antioxidant properties, making

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it necessary to consider the concentration of vitamers other than α-tocopherol before an assessment of the vitamin E and antioxidant activity of a sample can be made.

Separation and quantitation of the tocopherols in soybean oil have been achieved by various methods. Paper chromatography (Brown, 1952; Green et al., 1955; Ward, 1958; Herting and Drury, 1963), thin-layer chromatography (Herting and Drury, 1967), and more recently high-performance liquid chromatography (Van Niekerk, 1973; Carpenter, 1979; Cort et al., 1983; Rammell and Hoogenboom, 1985; Speek et al., 1985) have been used for this purpose with good results. Although a variation in total and individual tocopherol content from one sample of soybeans to another has been observed, there has not been an attempt to determine whether varietal differences exist or whether the observed variation is due to other factors. Varietal differences have been reported for corn (Combs and Combs, 1985) and winged beans (de Lumen and Fiad, 1982) recently.

In this paper, we report the content of the tocopherol vitamers in five soybean varieties grown under identical conditions. We also have examined the vitamin E content of tofus made from these varieties and the loss of vitamin E during the refrigerated storage of tofus from two of the soybean varieties.

MATERIALS AND METHODS

Soybeans. Five soybean varieties (Corsoy, Strayer, Vinton, Weber, 2325) were donated by Strayer Seed Co., Hudson, IA. The varieties were all grown in Iowa in the summer of 1984. The seeds were ground with a coffee mill to pass a No. 60 sieve. Four grams of ground beans was extracted with 100 mL of ethanol in a stoppered flask, under gold fluorescent light, for 30 min. The suspension was filtered through Whatman filter paper No. 4 and the residue reextracted with 50 mL of ethanol for 1 h. The ethanol extracts were combined and dried in a rotary vacuum evaporator at 40 °C. The extract was rinsed out of the round-bottom flask with two 25-mL protions of hexane and filtered through anhydrous Na_2SO_4. The hexane was evaporated under vacuum at 35 °C and the extract rinsed out with three 1-mL portions of isooctane into 10-mL graduated test tubes. The samples were centrifuged in a bench top centrifuge for 10 min.

Tofu Manufacture. Tofu was made by following the procedure of Johnson (1984). Nine hundred grams of soybeans was soaked in tap water for 10–12 h at room temperature. The hydrated beans were drained, combined with 6 L of tap water, and then ground to a slurry with a Cherry-Burrell Vibroreactor. The slurry was transferred to a steam-jacketed kettle, and an additional 1 L of tap water was added. The slurry was brought to 95 °C and stirred constantly. After 7 min, the cooked slurry was poured into a coarse-mesh filtering sack. One liter of water was added to the residue, and the combined filtrates were filtered through a fine cloth. A 30-mL aliquot of soy milk was removed to measure the solids content by using the light-scattering technique of Johnson and Snyder (1978). The solids content of the soy milk was then adjusted to the desired solids level by using tap water. The soy milk was brought up to 85 °C, and calcium sulfate was added for coagulation at this point. The amount of calcium sulfate added was such that its concentration in the soy milk would be 0.019 N. After settling for 5 min, the resulting coagulum was cut and poured into a cheesecloth-lined stainless-steel box with perforations on all sides to allow drainage. After it was pressed for 15 min, the tofu was placed in a water-filled plastic container and stored at 4 °C until tested.

Tofu Extraction. Forty grams of finely chopped tofu was blended with 100 mL of ethanol for 30 s. The resultant slurry was filtered through four layers of cheesecloth and a Whatman filter paper No. 4 under vacuum. The residue was extracted with 100 mL of ethanol, and the filtrates were combined and dried under vacuum at 40 °C. The remaining steps were identical with the ones described for the beans.

Ground soybeans and tofus were analyzed for moisture by drying in a convection oven at 70 °C for 18 h. Lipid content was determined in dried samples with hot hexane for 4 h in a Goldfish extraction apparatus. Moisture content ranged from 7.9% to 9.4% for the soybeans and from 74.0% to 81.3% for the tofus, respectively. Lipid content ranged from 22.5% to 25.9% for the soybeans and from 24.6% to 26.0% for the tofus, respectively (dry weight basis).

Storage Study. Tofus made from the varieties 2325 and Vinton were stored in a display case at 5 °C. Triplicate samples were taken after 0, 2, 4, 6, 9, 12, and 15 days of storage.

High-Performance Liquid Chromatography (HPLC). The chromatographic system consisted of a normal-bonded phase 250 × 4.6 mm Ultrasil NH_2 (Beckman) column with a 3-cm amino guard column (Brownlee Labs). The mobile phase was 1% 2-propanol in hexane at a flow rate of 2.0 mL/min. Twenty-microliter samples were injected by using a 20-μL loop. α-Tocopherol and γ-tocopherol were purchased from Eastman Organic Chemicals (Rochester, NY). δ-Tocopherol was isolated from commercial soybean oil. The soybean oil was extracted twice with 100% ethanol in a ratio 1:1 oil to ethanol. The ethanol was evaporated under vacuum, and the dried extract was dissolved in methanol. For separation and purification, a preparative reversed-phase HPLC system was used. The system consisted of a 250 × 9.4 mm Partisil ODS-3 C_{18} (Whatman) semipreparative column with a mobile phase of 3% water in methanol. Total chromatographic analysis time was 12 min. The technique of "heart cutting" was used for the collection of the peaks. A minimum of three passes through the system was necessary before δ-tocopherol of acceptable purity could be obtained.

The tocopherols were monitored by their absorbance at 280 nm with a fixed-wavelength UV detector (Beckman/Altex) equipped with one of two flow cells: an analytical cell for analytical work and a preparative cell for collection of compounds. Purity of the compounds used as standards was determined by using silica gel TLC with chloroform as the mobile phase, analytical HPLC, and spectrophotometric scans.

For analysis of recovery efficiency, standards dissolved in ethanol were added to the ground beans and the tofus before extraction. Recoveries for α-, γ-, and δ-tocopherol were 101, 104, and 100% from the ground beans and 94, 107, and 104% from the tofus, respectively.

RESULTS AND DISCUSSION

Large-scale separation of α- and δ-tocopherols from a synthetic mixture of tocopherols has been achieved by HPLC using a preparative silica column (The Supelco Reporter, 1984). Isolation of δ-tocopherol in milligram quantities from a natural source requires higher resolution than is necessary for a mixture of pure tocopherols because of the presence of other tocopherols and interfering compounds. In soybean oil, separation of γ-tocopherol (present in larger quantities than α-tocopherol) from δ-tocopherol is the critical purification step. γ-Tocopherol has a more similar polarity to δ-tocopherol than does α-tocopherol.

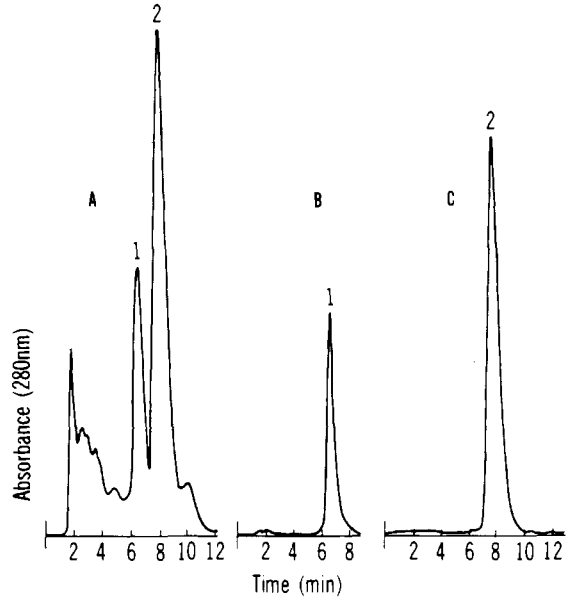

Figure 1. Isolation of δ-tocopherol (peak 1) and γ-tocopherol (peak 2) by preparative reversed-phase HPLC. Part A shows an ethanol extract of commercial soybean oil. Chromatograms B and C correspond to isolated δ- and γ-tocopherols, respectively, on the reversed-phase preparative column.

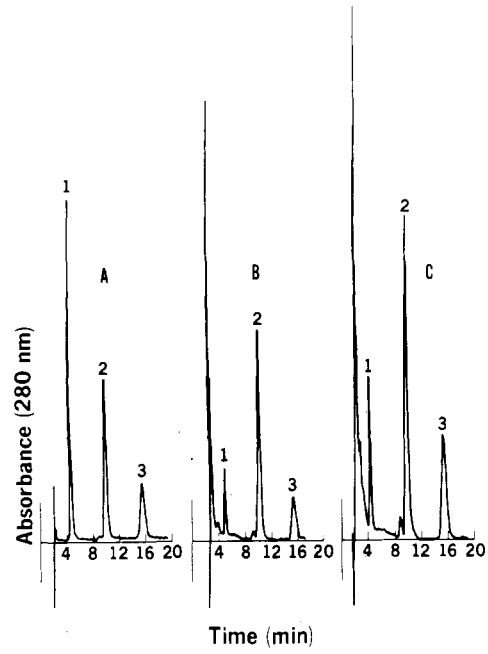

Figure 2. Normal-phase (amino) HPLC separation of α- (peak 1), γ- (peak 2), and δ-tocopherol (peak 3) in a mixture of pure tocopherols (A), an extract from ground soybeans (B), and an extract from tofu (C).

Figure 1A shows the chromatogram of an ethanol extract from commercial soybean oil. Two major peaks occupy most of the area under the chromatogram. Peaks 1 and 2 were collected, purified, and identified as δ- and γ-tocopherol, respectively. Parts B and C of Figure 1 are chromatograms of the tocopherols during the last heart-cut step.

The first HPLC system for the separation of tocopherols involved a silica column with hexane/diisopropyl ether as the mobile phase (Van Niekerk, 1973). Since then, other eluant mixtures for silica columns as well as the more stable reversed-phase systems have been employed with good results. Compared with silica columns and reversed-phase systems, normal-bonded phase systems exhibit a better stability and higher resolution of tocopherols (Westerberg et al., 1981), respectively. Chromatograms of a mixture of standards (Figure 2A), of an extract from ground soybeans (Figure 2B), and of an extract from tofu (Figure 2C) reveal satisfactory resolution of the three main tocopherols. α-Tocopherol tends to be eluted early and adjacent to a large peak containing UV light-absorbing compounds of nonpolar nature. Another tocopherol that has been found in soybeans in amounts of about 1% of the total tocopherol is β-tocopherol (Rammell and Hoogenboom, 1985). The small peak eluting earlier than γ-tocopherol probably is β-tocopherol according to the retention time of this compound in polar-bonded HPLC systems similar to the one described herein (Matsuo and Tahara, 1977; Jansson et al., 1980; Westerberg et al., 1981; Rammell and Hoogenboom, 1985). Because of its minimal presence in our soybean varieties, quantitation of β-tocopherol was not included in the present work. For purposes of calculating the total vitamin activity of a sample, however, β-tocopherol is considered half as active as α-tocopherol and 5 times as active as γ-tocopherol (National Research Council, 1980). In wheat germ, β-tocopherol may be as much as 40% of the total tocopherol content (Quaife, 1948).

In the preparation of foods and feedstuffs for analysis, lipid extraction with hot solvent generally followed by saponification using heat, KOH, and an antioxidant used to be a widespread practice. Extraction at room temperature with nonpolar solvents has been used lately in soybeans (Priestley et al., 1980) and in winged beans (de Lumen and Fiad, 1982). It has been proposed that some of the vitamin E in foods is bound up as a protein complex (Voth and Miller, 1958). With the use of a polar solvent such as ethanol, the investigator has more assurance of extracting all E vitamers. Saponification is used to cleave triglycerides and remove impurities of a reducing nature (Contreras-Guzman and Strong, 1982). But, the tocopherols are very unstable in the presence of alkali, particularly if oxygen is present (Ames, 1967). Saponification may be necessary when a sample contains tocopheryl esters, and gas chromatography with fluorescent detection is used for analysis (Van Niekerk, 1982). The presence of triglycerides does not seem to offer interference in the analysis of tocopherols by HPLC. For vegetable oils, injection of a few microliters dissolved in the mobile phase or another appropriate solvent has become accepted practice (Van Niekerk, 1973; Carpenter, 1979; Rammell and Hoogenboom, 1985). A similar approach on extracts from legume beans in which no added tocopheryl esters exist has been applied successfully (de Lumen and Fiad, 1982). Speek et al. (1985) have recently reported the absence of esterified E vitamers in several seed oils including soybean oil. Our extraction procedure was developed with the simplicity required for routine analysis. We took into

Table I. Tocopherol Content[a] and Vitamin E Activity[b] of Five Soybean Varieties and Tofus[c]

	α-tocopherol		γ-tocopherol[d]		δ-tocopherol		vitamin E act.	
variety	beans	tofu	beans	tofu	beans	tofu	beans	tofu
Vinton	28.4[a]	30.5[a]	171	187	48.7[b,c]	61.1[b]	46.0[c]	49.8[b]
2325	10.9[b]	19.7[a,b]	145	217	35.5[c,d]	64.3[b]	25.9[a]	42.0[a]
Corsoy	12.4[b]	10.5[b]	161	153	24.6[d]	35.8	28.7[a]	26.1
Weber	25.9[a,b]	22.5[a,b]	191	215	60.6[a,b]	76.9[a,b]	48.7[b,c]	44.8[a]
Prize	27.7[a]	32.6[a]	150	156	72.6[a]	85.8[a]	43.4[b]	49.1[b]

[a] Tocopherol content (μg/g of dry matter). [b] Vitamin E activity (mg of α-tocopherol equivalents/kg of dry matter). [c] Means in a column followed by a common letter are not significantly different at the 5% level. [d] No significant differences at the 5% level were found for γ-tocopherol means.

Table II. Total Milligrams of Tocopherols in 900 g of Starting Soybeans and in the Resulting Tofus

variety	vitamer	soybeans	tofu	% retentn
2324	α-tocopherol	9.0	7.0	78
	γ-tocopherol	119.4	76.9	64
	δ-tocopherol	29.2	22.8	78
Vinton	α-tocopherol	23.4	12.2	52
	γ-tocopherol	140.5	75.0	53
	δ-tocopherol	40.1	24.5	61

account the sample size required, time involved, amount of solvent, diversity, and amount of reagents and number of extraction steps.

Table I lists the tocopherol contents of the five soybean varieties analyzed and of the tofus made from soybeans of these varieties. The content of the three individual tocopherols is significantly different (99% confidence limit) among ground soybeans of the five varieties analyzed and among their tofus.

A rigorous comparison is not possible using most values in the literature because either the analyses are performed in refined, commercial oil or no reference is given as to the origin of the oil. Our values, nonetheless, agree with most of the previous reports on the tocopherol content of soybean oil (Brown, 1952; Green et al., 1955; Ward, 1958; Herting and Drury, 1963; Herting and Drury, 1967; Carpenter, 1979; Cort et al., 1983). Refined corn oil contained approximately half as much α-tocopherol and one-fourth as much total tocopherol as the lipid from freshly extracted seed corn. (Herting and Drury, 1963). Refined safflower oil, on the other hand, had α-tocopherol and total tocopherol levels similar to crude oil.

Tofus are better sources of tocopherol than ground soybeans on a dry basis, reflecting the changes in composition undergone by the beans as they are transformed into tofu. When the soy milk is obtained, the material that is retained by the coarse and fine filters consists mainly of seed hulls. These structures are likely to contain smaller amounts of any vitamin than the internal structures of the soybean seeds.

The loss in tocopherols caused by the processing of soybeans into tofu is described in Table II. As much as 50% of the tocopherols originally present in the soybeans may be lost in the process. Miller et al. (1952) reported the retention of some nutrients in commercially prepared soybean curd. Retention of thiamin was 13–25%; riboflavin, 15–27%; and niacin, 18–47%. Tocopherol retention in our study ranged from 52 to 78%. As a fat-soluble vitamin, it is expected that vitamin E will be lost to a lesser degree than the water-soluble vitamins when the newly formed curd is drained and pressed. The effects of food processing and storage on total tocopherols (Harris, 1962) and specifically on α-tocopherol (Ames, 1967) in foods have been described in some detail. Some of the processes in which a significant tocopherol loss has been determined are the polishing of rice, the milling and bleaching of wheat flour, the manufacture of breakfast cereals, and the canning of beans, corn, and peas (Ames, 1967). The tocopherols are unstable in the presence of unsaturated fats, oxygen, alkalis, ultraviolet light, and metal ions (Nelis et al., 1985). Wet heating at 80 °C for 4 h had no effect on the tocopherols of wheat flour, but dry heat for 4 h at 150 °C caused measurable destruction (Harris, 1962). The loss in tocopherols observed in the manufacture of tofu may be attributed mainly to the discarding of tocopherol-containing structures and partly to the peroxidation of the unsaturated oils and the contact with oxygen and metal ions (soybeans are high in zinc, iron, and copper) during the heating steps.

The vitamin activity expressed as milligrams of α-tocopherol equivalents per kilogram of dry matter of ground beans or tofu is shown in Table I. To calculate vitamin activity, the milligrams of γ-tocopherol were multiplied by 0.1 (National Research Council, 1980) and those of δ-tocopherol by 0.01 (Bieri and Mckenna, 1981), and the sum was added to the milligrams of α-tocopherol. Even with only 10% of the biological activity of α-tocopherol, γ-tocopherol contributes 34–59% of the vitamin E activity of the soybean varieties analyzed.

The determination coefficients of the linear regression analysis of the plots of tocopherol content (μg/g on a dry basis) vs. time (days) of storage of tofu were all not statistically significant ($P < 0.05$). Priestley et al. (1980) aged mature soybean seeds by "natural aging" (long-term storage) and by "accelerated aging" (exposure to high temperature and humidity). The levels of the three main tocopherols (α, γ, δ) and the levels of free radicals were fairly constant after 8 years of natural aging and 4 days of accelerated aging. The manufacture of tofu involves several processes that will result in a rearrangement of the seed structural components, with consequent exposure of internal structures to the environment. In addition, considerable heating is employed, and calcium is added in the form of calcium sulfate. It seems, however, that, under the conditions of storage utilized, the tocopherol content of tofu is not significantly affected after a period of 2 weeks.

Kodicek et al. (1959) reported retention of 60% of the α-tocopherol and one-third of the total tocopherol of corn after storage for 12 weeks at room temperature. In tortilla prepared from steeped corn in 1% lime water, 95% of the tocopherol was destroyed after the same storage treatment. The stable content of tocopherols during storage of tofu may be attributed to the short storage time and to the environment of reduced oxygen pressure in which the product is held.

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Registry No. α-Tocopherol, 59-02-9; γ-tocopherol, 7616-22-0; δ-tocopherol, 119-13-1.

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Characterization of Bovine Urine and Adipose Interlaboratory Performance Evaluation Samples Containing Biologically Incorporated Chlorophenols[1]

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In vivo incorporated bovine urine and adipose reference materials were prepared by oral administration of Lindane, 1,2-dichlorobenzene, 2,4-dichlorophenol, 1,2,3,4-tetrachlorobenzene, and pentachlorophenol to cows. The tissues and fluids obtained were homogenized and analyzed for administered compounds and major metabolites. Virtually all of the 20-g dose of 2,4-dichlorophenol was eliminated in the urine within 24 h following administration, and 70% of the Lindane was metabolized to tri- and tetrachlorophenols and excreted in the urine. The rest of the administered Lindane (25%) was deposited unmetabolized in the adipose. Half of the administered tetrachlorobenzene was deposited in the adipose tissue. The urine samples were found to be reasonably stable with chlorophenol concentrations remaining constant over four freeze/thaw cycles. Bovine urine and adipose reference materials are now available to laboratories to facilitate data comparison or to test analytical procedures for these environmentally important toxicants and their metabolites.

INTRODUCTION

Chlorophenols are of interest because of their inherent toxicity, the presence of dioxin impurities in chlorophenols, and their wide distribution in the environment (Lee and Chau, 1983). Chlorophenols have been disseminated through their direct use as fungicides or bactericides and as breakdown products of a wide variety of pesticides (Cremyln, 1978). Despite extensive analysis and study, no single method has emerged as a standard analytical technique for determining chlorophenols in biological tissues and fluids. Some of the techniques that have been used for the analysis of chlorophenols include: GC/ECD determination of underivatized chlorophenols (Kalman, 1984), GC/ECD determination of their derivatives (Edgerton, 1981), GC/MS (Hargesheimer and Coutts, 1983), HPLC of underivatized chlorophenols with electrochemical (McMurtrey et al., 1984) or UV detection (Pelsari and Aitio, 1982), and HPLC of derivatives with fluorescence detection (Carlson et al., 1984). As there are no definitive studies of the accuracy of these various methods, meaningful comparison of analytical results reported in the chemical literature is a difficult proposition.

In addition to the data comparability problems caused by using different analytical methods, chlorophenol analyses are complicated by the fact that these compounds

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