Determination of Bensulfuron Methyl Residues in Rice Grain and Straw by High-Performance Liquid Chromatography

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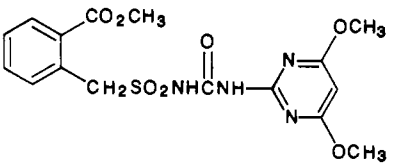
High-performance liquid chromatographic methods using photoconductivity detection were developed to determine residues in rice grain and straw of bensulfuron methyl, methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate, the active ingredient in Du Pont Londax rice herbicide. By these methods, bensulfuron methyl was extracted from rice grain in methylene chloride or from rice straw in methylene chloride containing 0.5% acetic acid. Solvent partitioning and solid-phase extraction were used to separate bensulfuron methyl from major interfering sample components prior to determination by normal-phase high-performance liquid chromatography (HPLC) using a photoconductivity detector. For grain and straw, respectively, detection limits (based on calibration curves) were 0.02 and 0.05 ppm and recoveries averaged 97% and 88%. No residues were detected in excess of the detection limits in 121 samples of rice grain from field plots treated at up to 400 g of ai/ha nor in 21 samples of rice straw from field plots treated at up to 140 g of ai/ha.

Du Pont Londax rice herbicide is a broad-spectrum product for preemergence or early postemergence control of most broadleaf weeds and sedges in transplated or direct-seeded paddy rice. Best results are obtained by a single early postemergence broadcast application to the flooded paddy at 20–50 g of ai/ha for annual broadleaf weeds and sedges and at 40–100 g of ai/ha for perenial broadleaf weeds and sedges. Londax is less active on grasses.

The active ingredient of Londax rice herbicide is bensulfuron methyl, methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate. Bensulfuron methyl, formerly designated by the code name DPX-F5384, has the following structural formula:



The chemical and physical properties, herbicidal efficacy, crop tolerance, and mammalian toxicology of bensulfuron methyl were reported by Du Pont in the Londax Technical Bulletin (undated) and by Takeda et al. (1985). The mode of herbicidal action was also reported by Takeda et al. (1986).

This paper describes the analytical methods developed and used to determine residues of bensulfuron methyl in rice grain and straw in support of Londax registration. By these methods, bensulfuron methyl was determined in sample solutions by high-performance liquid chromatography using a photoconductivity detector.

The basis of the photoconductivity detector is electrical conductance measurement of ions formed by dissociation of noncharged sample components in the mobile phase following irradiation with ultraviolet light. After the sample components are separated by the chromatographic column, they enter the photoconductivity detector in the mobile-phase stream. The mobile-phase stream is split into two separate streams that have equal flow rates and pass through tubing of equal diameters and lengths to the analytical and reference cells, respectively, where the electrical conductance of each stream is measured. The streams differ in only one respect: Immediately before entering the conductivity cell, the analytical stream passes through a quartz coil and is irradiated by ultraviolet light from a mercury lamp. The reference stream is not irradiated. Energy from the ultraviolet lamp causes a fraction of the bensulfuron methyl and a few other ionizable sample components in the analytical stream to ionize. The increased conductance of the analytical stream compared to the reference stream is recorded as the detector signal.

EXPERIMENTAL SECTION

Chemicals and Reagents. The bensulfuron methyl reference standard was synthesized, purified, and assayed to be 99.5% pure in the Du Pont Agricultural Products Department, Research Division Laboratories. All organic solvents used for sample extraction, cleanup, and liquid chromatography were either HPLC grade obtained from J. T. Baker Chemical Co. or high-purity grade obtained from Burdick and Jackson Laboratories. Deionized water was used for these methods. Chlorinated water must not be used because residual chlorine may cause decompositon of bensulfuron methyl.

A 100 μg/mL stock standard of bensulfuran methyl in methylene chloride was used to prepare a 1 μg/mL standard in methylene chloride for fortification purposes and a 1 μg/mL standard in mobile phase for HPLC calibration. Both 1 μg/mL standards were prepared daily by pipeting 1 mL of stock standard solution into a volumetric flask, evaporating the solvent with a gentle stream of dry nitrogen, and making up to 100-mL volume with either methylene chloride or HPLC mobile phase. HPLC standards of 0.10–1.0 μg/mL bensulfuron methyl in HPLC mobile phase were prepared by appropriate dilution of the 1 μg/mL HPLC standard.

Different mobile-phase compositions were used for analysis of grain and straw samples. The compositions of the HPLC mobile phases (mL) are given below:

component	grain	straw
n-hexane	750	720
isopropyl alcohol	125	110
methyl alcohol	100	110
acetonitrile	25	55
acetic acid	2	2
water	1	1

The HPLC column cleaning solution consisted of 400 mL of isopropyl alcohol, 100 mL of glacial acetic acid, and 10 mL of water.

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The pH 10 buffer solution for the straw analysis procedure was prepared by combining 50 mL of pH 10 commercial buffer solution (Fisher Scientific Co., Catalog No. SO-B-116) and 450 mL of water preadjusted by pH meter to pH 10 with 0.1 M sodium hydroxide solution.

Disposable C_{18} Bond Elut extraction columns (Analytichem International, Harbor City, CA; Part No. 607306) were used to collect and concentrate bensulfuron methyl from aqueous solution.

Apparatus. Bulk samples of rice straw were homogenized for analysis on a Hobart Commercial Food Cutter.

Samples of grain and straw were finely ground during solvent extraction using a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH) with Shaft No. SDT 182 EN.

Bensulfuron methyl analyses were performed on a Du Pont Model 850 HPLC instrument fitted with a 4.6 mm × 25 cm Du Pont Zorbax Sil column, a Tracor Model 965 photoconductivity detector with a mercury lamp, and a Hewlett-Packard Model 3380A integrating recorder. To permit accurate balancing of the mobile-phase flows through the reference and analytical cells of the detector, a metering value (Nupro Model SS-2SA-TFE) was installed on the reference cell discharge tube. The ion-exchange resin tube and the micropump of the detector were not used because deionization of the mobile phase is not necessary and because the resin could actually introduce contaminants into the mobile phase.

ANALYTICAL PROCEDURES

Sample Preparation for Rice Grain. Samples of rice grain from the United States agricultural test sites were frozen as soon as feasible after collection and were shipped to the laboratory in dry ice. Samples of rice grain from Thailand, Java, and The Philippines were shipped to the laboratory at ambient temperatures. The shipping temperature for the four samples of rice grain from Australia was not recorded. Upon arrival at the laboratory, all samples were stored at −20 °C until analyzed.

To determine bensulfuron methyl in whole or polished rice grain, each 25-g representative sample was extracted three times with 100-mL quantities of methylene chloride. Extractions were performed in a glass centrifuge bottle with use of a Tekmar Tissumizer to grind the rice grain to a fine powder. Typical grinding times were 60, 45, and 30 s for the three extractions. After each grinding operation, the sample was centrifuged and the extract was vacuum filtered through a sintered glass filter with prefilter. The combined extracts were evaporated to dryness on a rotary evaporator with the water bath at 35 °C.

Bensulfuron methyl was separated from hexane-soluble components by dissolving the sample extract residue in 50 mL of acetonitrile and washing the solution three times with 50-mL quantities of hexane in a separatory funnel. After being washed with hexane, the acetonitrile solution was evaporated to dryness on a rotary evaporator with water bath at 35 °C.

Additional cleanup of each grain sample was accomplished on disposalbe solid-phase extraction columns. A disposable C_{18} Bond Elut column was conditioned by flushing it with 25 mL of acetonitrile and then with 25 mL of 0.15 M aqueous ammonium hydroxide solution. The sample residue was dissolved in 50 mL of 0.15 M ammonium hydroxide, and the solution was passed through the column slowly so that the effluent formed distinct drops, not a steady stream. After the sample solution was passed through the column, the glassware that previously held the sample was rinsed with 10 mL of 0.15 M ammonium hydroxide, and this rinse solution was also passed through the column. Air was then drawn through the column to remove residual water. Bensulfuron methyl was eluted from the column with 10 mL of acetonitrile, and the eluate was evaporated to dryness under a gentle stream of dry nitrogen.

Samples of polished rice grain were analyzed for bensulfuron methyl as described in HPLC Analysis without further cleanup. Samples of whole rice grain required additional cleanup. The residue from each whole rice grain sample was redissolved in 50 mL of 0.15 M ammonium hydroxide, and the solution was washed once with 50 mL of toluene. The aqueous phase was then acidified with HCl to pH 4.0 with a pH meter to monitor the pH. Bensulfuron methyl was extracted from the acidified aqueous phase with two 50-mL quantities of toluene. The toluene extracts were combined and evaporated to dryness on a rotary evaporator with water bath at 40 °C. Bensulfuron methyl was determined in samples of whole rice as described in HPLC Analysis.

Sample Preparation for Rice Straw. Samples of rice straw from the United States agricultural test sites were frozen as soon as feasible after collection and were shipped to the laboratory in dry ice. The shipping temperature of the four straw samples from Australia was not recorded. Upon arrival at the laboratory, all samples were stored frozen at −20 °C until analyzed. Bulk samples of straw were homogenized before analysis by cutting the straw to lengths of less than 1 cm with a commercial food cutter. Powdered dry ice was added to each sample during this operation to facilitate cutting and to minimize sample decomposition. Dry ice was allowed to sublime from each homogenized sample in a loosely capped bottle to prevent moisture condensation.

To determine bensulfuron methyl in rice straw, each 10-g sample of homogenized straw was extracted three times with 0.5% acetic acid in methylene chloride with 135, 100, and 100 mL, respectively, for the three extractions. Extractions were performed in a glass centrifuge bottle with a Tekmar Tissumizer to grind the straw to a fine powder. The grinding operation for the first extraction was simplified by allowing the straw to soak for 5–10 min in extraction solvent to absorb solvent and reduce the tendency of the straw to float. For the first extraction, the Tekmar Tissumizer was operated until all the straw was ground to lengths of ≤1 mm. For the second and third extractions, typical grinding times were 30–60 s. After each grinding operation, the sample was centrifuged, and the extract was vacuum filtered through a sintered glass filter with prefilter. The combined extracts were evaporated on a rotary evaporator with water bath at 35 °C until only residue of acetic acid remained. Most of the acetic acid was then evaporated with a gentle stream of dry nitrogen, but the sample residue was left damp to facilitate subsequent dissolution. If the acetic acid was completely evaporated, the sample residue adhered to the flask and made the subsequent dissolution difficult.

Bensulfuron methyl was separated from hexane-soluble components by acetonitrile–hexane partitioning. To dissolve the sample residue, 75 mL of acetonitrile and 75 mL of hexane were measured out, and small portions of the solvents were used alternatively. After the acetonitrile–hexane mixture was transferred to a separatory funnel, the 75-mL acetonitrile phase was washed three times with 75-mL quantities of hexane. Samples were centrifuged after each wash to ensure complete phase separation. After the hexane washes, the acetonitrile solution was evaporated to dryness on a rotary evaporator with water bath at 35 °C. Care was taken to make certain that the sample was completely dry because residual acetic acid would interfere

with subsequent procedural steps.

Additional cleanup was accomplished by solvent partitioning between water and methylene chloride and then water and toluene as follows. The sample residue was dissolved in 10 mL of methylene chloride, and the solution was transferred to a centrifuge bottle. The sample flask was rinsed with 200 mL of pH 10 buffered water (see Chemicals and Reagents for composition) to recover traces of bensulfuron methyl, and the rinse solution was added to the methylene chloride in the centrifuge bottle. After the solution was shaken vigorously for 3 min and centrifuged, the aqueous phase was carefully removed by pipet or syringe, being careful to leave the methylene chloride and the flocculent interface undisturbed. At pH 10, bensulfuron methyl was retained in the aqueous phase.

During the methylene chloride–water partitioning step, the pH of the aqueous phase must remain at 10 and the volume of methylene chloride must be much smaller than the volume of aqueous phase to prevent loss of bensulfuron methyl into the methylene chloride. After confirming by pH meter that the aqueous phase had remained at 10, the methylene chloride wash solution and flocculent interface were discarded, and the aqueous phase was washed with a second 10-mL quantity of methylene chloride and centrifuged, and the aqueous phase retained.

The aqueous phase was then washed once with 100 mL of toluene by shaking the solvent mixture vigorously for 3 min and separating phases by gravity or centrifugation. The toluene phase was discarded. After the toluene wash, the aqueous phase was acidified to pH 4.0 by slowly adding 0.1 M HCl with stirring while the pH was monitored with a pH meter. Bensulfuron methyl was extracted from the acidified aqueous phase with two 100-mL quantities of toluene. For each extraction the mixture was shaken vigorously for 2 min, and phases were separated by gravity or centrifugation. The two extracts were combined, five drops of glacial acetic acid were added, and the solution was evaporated to dryness on a rotary evaporator with water bath at 40 °C. Residual toluene or acetic acid was evaporated from the sample flask using a gentle stream of dry nitrogen. Bensulfuron methyl was determined in the samples of rice straw as described in HPLC Analysis.

HPLC ANALYSIS

Bensulfuron methyl was determined in rice grain and straw samples by high-performance liquid chromatography using a photoconductivity detector by comparing the chromatographic peak heights for bensulfuron methyl in the sample solutions with the corresponding peak heights for standard solutions containing known quantities of bensulfuron methyl. The chromatographic column and mobile-phase compositions were reported in Chemicals and Reagents. Residues of grain and straw were dissolved in 5 mL of their respective mobile phase for analysis. Chromatographic conditions for bensulfuron methyl analysis were as follows: column temperature, 35 °C; mobile phase flow rate, 1.0 mL/min; injection volume, 10 μL; retention volumes, 7.4 mL for grain analyses, 7.3 mL for straw analyses.

Standard curves of peak heights versus nanograms injected were linear with zero intercept for injections of up to 25 ng of bensulfuron methyl. The analytical sensitivity for bensulfuron methyl was typically 70–250 mm/ng when the detector was operated at maximum sensitivity, and the detector response was recorded on a 1-mV recorder with 167-mm full-scale deflection.

Care was taken to make certain that the Zorbax Sil column was properly conditioned and equilibrated with the HPLC mobile phase before analysis. If the column was not property conditioned, low sensitivity or drifting sensitivity was experienced. The column was conditioned by pumping the previously described HPLC cleaning solution through the entire system at 0.5 mL/min for at least 4 h. HPLC mobile phase was then pumped through the system at 0.5 mL/min for at least 4 h to establish equilibrium between the column and mobile phase.

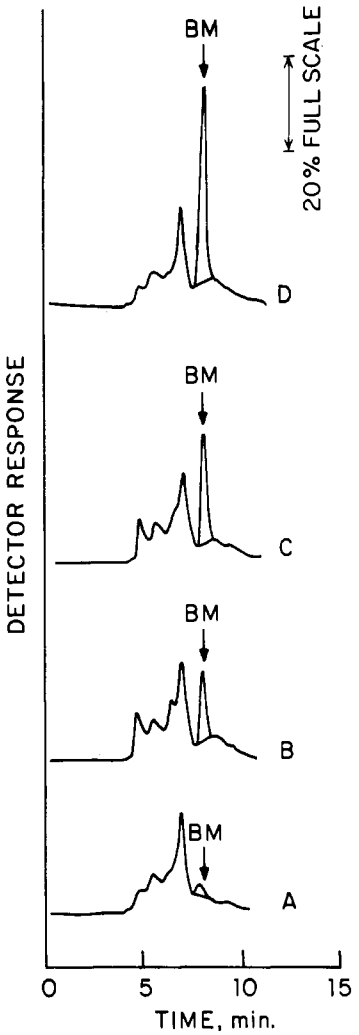

Figure 1. Representative chromatograms for determination of bensulfuron methyl in rice grain. BM denotes bensulfuron methyl. Key: (A) unfortified control; (B) control fortified at 0.02 ppm, 110% recovery; (C) control fortified at 0.04 pm, 99% recovery; (D) control fortified at 0.08 ppm, 95% recovery.

RESULTS AND DISCUSSION

Representative chromatograms for the determination of bensulfuron methyl in rice grain and straw samples are shown in Figures 1 and 2. These chromatograms are for unfortified control samples and for control samples fortified with bensulfuron methyl before analysis at the detection limit and at higher fortification levels. Chromatograms for all grain and straw samples from field plots treated wtih Londax rice Herbicide were indistinguishable from those of control samples. These chromatograms demonstrate the highly specific response of the photoconductivity detector for sulfonylurea compounds. The bensulfuron methyl peaks of Figures 1 and 2 would have been totally obscured by full-scale recorder deflection if a 254-nm ultraviolet absorbance detector had been used.

The detection limit for HPLC determination of bensulfuron methyl was 1 ng at the 95% confidence level by the method of Hubaux and Vos (1970). By this method, confidence limits were calculated for the standard curve,

Table I. Recovery Data for Bensulfuron Methyl In Rice Grain and Straw

sample type	analyst	% recovery[a] 0.02 ppm	0.04 ppm	0.05 ppm	0.08 ppm	0.10 ppm	0.20 ppm	0.50 ppm
polished rice grain	1	100	90		100			
		96	88		100			
		98	110		120			
		92	130		95			
		110	110					
		110	99					
whole rice grain	1	100	94		95			
		100	100		90			
		100	74		110			
		98	95		100			
		94	120		120			
		110	130		130			
			76					
			120					
whole rice grain	2		67	80		67	85	110
			75	68		72	75	
			80			78		
rice straw	1			96		110	84	
						89		
rice straw	2					70	80	
						67		
						95		
						70		
						110		
						100		

[a] Each value is the result of a single experiment.

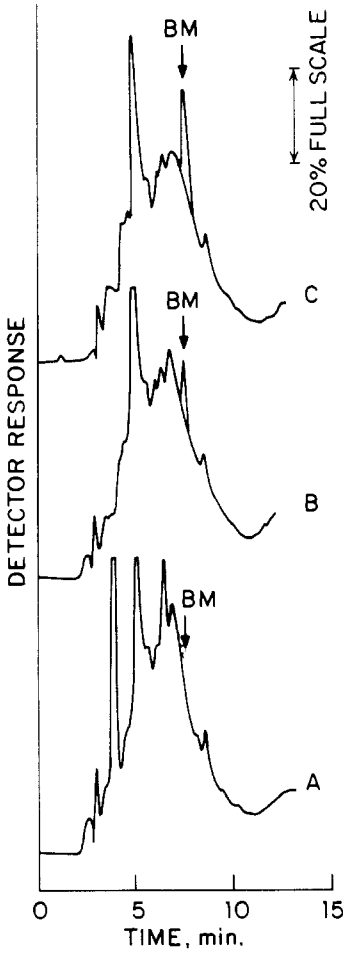

Figure 2. Representative chromatograms for determination of bensulfuron methyl in rice straw. BM denotes bensulfuron methyl. Key: (A) unfortified control; (B) control fortified at 0.05 ppm, 96% recovery; (C) control fortified at 0.10 ppm, 89% recovery.

and the detection limit was evaluated from those limits. A detection limit of 1 ng for the HPLC analysis corresponds to a detection limit of 0.02 ppm for 25-g grain samples and 0.05 ppm for 10-g straw samples.

Table II. Treatment Rate and Preharvest Interval for Field-Treated Rice[a]

bensulfuron methyl treatment rate, g ai/ha	preharvest interval, days from last field treatment to harvest
untreated	38 control samples analyzed
25	156
40	95, 95, 110, 110, 112, 112, 114, 114
50	81, 81, 95, 130, 156
57	120, 120
70	120, 120, 120, 120, 125, 126, 126, 129, 146, 146
75	112, 112, 114, 114, 123, 123, 124, 124, 142, 142, 153, 153, 153, 153, 156
76	120, 120, 120, 120
80	95, 95, 110, 110
100	95, 130
105	120
120	135
140	120, 120, 120, 120, 125, 126, 126, 129, 146, 146
150	123, 123, 124, 124, 127, 127, 153, 153, 153, 153
200	81, 81, 93, 93, 95, 123, 123, 130
210	120
400	95

[a] All samples of rice grain contained <0.02 ppm bensulfuron methyl.

A control sample and at least one control sample fortified before analysis with a known quantity of bensulfuron methyl were analyzed with every set of four to six field-treated samples to demonstrate the absence of interferences, to provide a check of recovery efficiency, and to confirm that the retention time of benzulsufuron methyl in the sample matrix was consistent with that of the standards. Standards were analyzed frequently during each series of sample analyses to confirm the stability of the instrument response and retention time.

Recovery efficiencies were determined on rice grain control samples fortified before analysis at the detection limit and at several higher fortifications levels to 25 times the detection limit. Recovery efficiencies were determined

Table III. Treatment Rate and Preharvest Interval for Field-Treated Rice[a]

bensulfuron methyl treatment rate, g ai/ha	preharvest interval, days from last field treatment to harvest
untreated	6 control samples analyzed
25	156
50	156
70	123, 126, 127, 146
75	156
76	110, 110, 120, 120
140	123, 126, 127, 146

[a] All samples of rice straw contained <0.05 ppm bensulfuron methyl.

similarly on rice straw control samples fortified at 1, 2, and 4 times the detection limit. The recovery data are reported in Table I for grain and straw samples analyzed by two analysts working independently on separate equipment. For the 47 rice grain recovery samples, the mean recovery efficiency was 97% with a standard deviation of 17%. For the 11 rice straw recovery samples, the mean recovery efficiency was 88% with standard deviation of 16%.

Bensulfuron methyl residues were determined in 121 rice grain samples from agricultural test plots in the United States, Thailand, Java, Australia, and The Philippines that had been field-treated with Londax rice herbicide at up to 400 g of ai/ha. None of the rice grain samples contained bensulfuron methyl residues in excess of the 0.02 ppm detection limit. These data are summarized in Table II by treatment rate and preharvest interval.

Bensulfuron methyl residues were determined in 21 rice straw samples from test plots in the United States and Australia that had been field-treated with Londax rice herbicide at up to 140 g of ai/ha. None of the rice straw samples contained bensulfuron methyl residues in excess of the 0.05 ppm detection limit. These data are summarized in Table III by treatment rate and preharvest interval.

The absence of detectable residues of bensulfuron methyl in rice grain and straw by these methods is consistent with the results of the metabolism study in which greenhouse-grown rice plants were treated with 14C-labeled bensulfuron methyl at 200 g of ai/ha. Analysis of the mature grain and straw for bensulfuron methyl by measurement of radioactivity 4 months after treatment of the rice plants showed 0.001 ppm in rice straw and 0.002 ppm in rice grain.

The bensulfuron methyl analytical methods have proven adequate for determination of the active ingredient of Londax rice herbicide in rice grain and straw with detection limits of 0.02 and 0.05 ppm, respectively. Residues of bensulfuron methyl were below the limits of detection in all of the grain and straw samples analyzed.

Registry No. Bensulfuron methyl, 104466-83-3.

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X-ray Fluorescence and Atomic Absorption Spectrophotometry Measurements of Manganese, Iron, Copper, and Zinc in Selected Foods

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A simultaneous multielement method for X-ray fluorescence (XRF) analysis was validated for Mn, Fe, Cu, and Zn in biological and food materials. The method uses the CEMAS approach to quantitation without similar standards. Average biases for nine NBS standards ranged from 0.7 ppm (2.2%) for Cu to 3.4 ppm (2.3%) for Mn, and relative standard deviations ranged from 2.5% for Zn to 9.2% for Fe. Detection limits averaged 0.6 ppm for Cu and Zn, 1.2 ppm for Fe, and 1.4 ppm for Mn (dry-weight basis). XRF and atomic absorption spectrophotometry (AAS) measurements were compared for 96 samples from different sources of 21 foods. Average biases between the XRF and AAS data ranged from −2.1 ppm for Cu to +0.4 ppm for Mn. Relative standard deviations ranged from 3.8% for Zn to 8.3% for Fe among sample aliquots and from 21% for Cu to 37% for Fe among different sources of the foods.

The average supermarket has 10000 or more food items (Kinder et al., 1984), and this number is continually increasing. As foods are raised under new agronomic conditions and processed with new procedures, it becomes a major challenge to provide up-to-date data on the mineral composition of the food supply. There is great need for rapid, accurate, multielement analytical methodologies that require minimal sample preparation. Protein, moisture, fiber, and oil are routinely analyzed by nondestructive methods in many foods and animal feeds (Norris, 1984; Hirschfeld and Stark, 1984; Park et al., 1982; Polesello and Giangiacomo, 1983). For mineral determination in foods, atomic absorption spectrophotometry (AAS) is most widely used (Harnly and Wolf, 1984; Ihnat, 1984) and usually requires dissolution of samples. For some elements, chemical interferences or matrix effects are still significant

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