

Study Title

Napropamide: Analytical Method For Determination and Validation
in Orange Processed Commodities

Data Requirement

EPA Guideline 40 CFR 171-4(c)

Author

Henry R. Storoni

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Performing Laboratory

ICI Americas Inc.
Western Research Center
Environmental Chemistry Section
1200 South 47th Street
Box 4023
Richmond, CA 94804-0023

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1 SUMMARY/INTRODUCTION

1.1 Scope

A method for the determination of napropamide residues has been validated for the following food commodities: oranges juice, orange molasses, orange oil, and orange dried pulp.

1.2 Principles

This method is intended for determining residues of napropamide at levels of 0.05 to 0.25 microgram(s) per gram in the processed products of orange juice, molasses, oil and dried pulp.

Napropamide residues are extracted from a measured weight of orange juice, molasses and dried pulp by blending the samples with toluene. The organic phase is filtered and traces of water are removed using a desiccant. The orange molasses and dried pulp sample extracts require another clean-up step with an alumina column for GC-NP analysis. All the toluene extracts are analyzed for napropamide by capillary gas chromatography with splitless injection and nitrogen/phosphorous (GC-NP) detection.

Napropamide residues from orange oil are extracted with acetonitrile, partitioned with two hexane washes, cleaned up with elution through an alumina column and the toluene extract analyzed like the other orange processed commodities.

Confirmation of unexpected peaks in the control or treated samples is done by capillary gas chromatography with mass selective detection (GC-MSD).

2 MATERIALS/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment capable of providing equivalent sensitivity and selectivity, and reagents of comparable purity can be used.

2.1 Equipment

2.1.1 Gas chromatograph. Hewlett-Packard Model 5880 designed for use with capillary columns, temperature programming of the column oven, and nitrogen phosphorus (NP) detector. The gas chromatograph is equipped with an HP 7672A automatic injector, a Series II plotter, and an HP LAS on-line data acquisition system (Hewlett-Packard Company, Palo Alto, CA).

2.1.2 Gas-chromatographic column. 15 m length by 0.53 mm i.d. fused silica capillary column with a 1.0 μm film thickness of 6 % cyanopropylphenyl (J & W, DB-1301 Cat.# 122-13).

2.1.3 Confirmatory system: gas chromatograph with mass selective detector. For the analysis of dried orange pulp, an HP 5890 II gas chromatograph, HP 5970 mass selective detector, and HP UNIX workstation was used. Column: HP-1; 12 m x 0.2 mm i.d. x 0.33 μm film thickness of cross-linked methyl silicone.

For the other processed commodities a Hewlett Packard HP5880A gas chromatograph was used. Column: J & W DB-17, 10 m x 0.2 mm i.d. x 0.33 μm film thickness of cross-linked 50 % phenyl 50 % methyl silicone (J & W Cat. # 122-1712). Hewlett Packard HP 5970 mass selective detector, and HP 9000 236 ChemStation computer and integration.

2.1.4 Gas-purification traps. Supelco in-line gas purifier and a J & W moisture trap for removing water and oxygen from the helium carrier gas (Supelco Cat. # 2-3800, J & W Cat. #016-1150).

2.1.5 Syringes. 10 μL capacity (Hamilton 701N) syringe for the autosampler and 100 μL and 250 μL capacity syringes (Hamilton 1700 series) for fortification.

2.1.6 Blender. Waring blender, and a glass Eberbach container #8470, 500 mL (VWR Cat. # 58981-009).

2.1.7 Filter paper. Whatman #2, Qualitative, 24 cm (Scientific Products Cat. # 2412-24).

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2.1.8Glass bottles. 2, 4 and 8 ounce narrow mouth bottles with Polyseal-lined screw-capped lids and 16 ounce wide-mouth bottles using aluminum liners.

2.1.9Reciprocating shaker. Eberbach Corporation, Ann Arbor, MI.

2.1.10Evapomixer. Buchler Instruments, EVAPO-MIX, capable of heating, vortexing and high vacuum of centrifuge tubes. (Buchler Instruments, Fort Lee, NJ)

2.1.11Separatory funnels. One or two sets of nine 60-mL glass separatory funnels equipped with appropriate stopcocks.

2.1.12Centrifuge tubes. One or two sets of nine Pyrex 50-mL centrifuge tubes with appropriate stopcocks and glass bridging to connect to the evapomixer.

2.1.13Chromatography column clean-up tubes. Nine tapered glass columns of dimensions 0.5 cm diameter x 25 cm length. (Bio-Rad, 3300 Regatta Blvd., Richmond, CA 94804; Cat. # 737-031)

2.2Reagents and Standards

2.2.1Solvents. Reagent grade or purer toluene, acetone, acetonitrile, hexane, and distilled water are acceptable. All solvents must be of high purity and suitable for use in trace organic analyses by gas chromatography.

2.2.2Acetonitrile:toluene. 1:3 (v/v).

2.2.3Acetone:toluene. 1:1 (v/v).

2.2.4Napropamide analytical reference standard. ASW 1079-R 99.8 % purity or equivalent, available from ICI Americas Inc., 1200 South 47th Street, Box No. 4023, Richmond, CA 94804-0023; Attention: Environmental Sciences Department Manager.

2.2.5Activated alumina. Neutral, 80-200 mesh. (Fischer Cat. # A540-500)

2.2.6Sodium sulfate. Granular, anhydrous, 12-60 mesh. (J. T. Baker Cat. # 3375-01)

2.2.7 Preparation of calibration and fortification solutions. Two napropamide stock solutions are prepared, a stock calibration solution, and a stock fortification solution. Calibration solutions are used to calibrate the instrument. Fortification solutions are used to fortify samples and demonstrate procedural recoveries. The stock calibration solution is prepared in toluene, and the stock fortification solution is prepared in acetone. To prepare these stock solutions, at nominal concentrations of 1000 µg/mL, place a known quantity (0.0500 g ± 0.0250 g) of napropamide reference standard of known purity into a clean 4-ounce narrow-mouthed glass bottle. Calculate the weight of solvent to add, based on the weight of reference standard taken, the purity of the reference standard, and the density of the solvent, as follows:

$$S = (W \times P \times D) / A$$

where S = the weight of solvent to add (g)

W = the weight of primary standard taken (mg std)

P = the purity of the primary standard (mg a.i./mg std)

D = the density of the solvent (g/mL)

A = the final analyte concentration desired (expressed as mg analyte/mL solvent: 1.0 mg/mL = 1000 µg/mL = 1000 pg/µL)

Use toluene (D = 0.867 g/mL) for calibration solutions, and acetone (D = 0.792 g/mL) for fortification solutions. Add a sufficient, quantitative weight of the appropriate solvent to the bottle. Cap the bottle with a Polyseal-lined cap, mix the contents thoroughly, and store in a freezer at -20° C when not in use.

To prepare working standard solutions for fortification purposes, dilute the stock fortification solution with acetone, by volume or weight, to give 10 and 100 µg/mL solutions. To prepare a working standard solution for calibration purposes, dilute the stock calibration solution with toluene to give 0.1, 0.025, 0.05, and 0.01 µg/mL solutions.

2.3 Analytical Procedure

2.3.1 Preparation of orange juice, molasses, oil, and dried pulp.

Orange samples were processed by the University of Florida according to ICIA Study Protocol NAPR-91-PR-03. The processed orange products were frozen within two hours of collection.

Bulk samples are stored at $-20 \pm 10^{\circ}\text{C}$ with temperatures monitored in the original shipping containers. A freezer alarm system was used to monitor compliance with the specified temperature limits.

2.3.2 Extraction of the orange juice, molasses, dried pulp, and oil.

Orange juice: Weigh a 30.0 g (± 0.1 g) subsample of the orange juice directly into the blender jar and let it thaw.

Add 120 mL (or 104 g) of toluene into the blender jar with a graduated cylinder or laboratory-calibrated pipette. Seal the top of the blender jar with aluminum foil, screw the cap on tightly, and blend the contents in the Waring Blender at moderate speed for three minutes.

No emulsions were formed in any orange juice extracts. However, if emulsions are formed, they may be broken by centrifugation, addition of sodium chloride, freezing, or other appropriate techniques. Any steps taken to break the emulsions must be recorded.

Filter the unemulsified extracts of the orange juice through 25 to 30 g of anhydrous sodium sulfate contained in the filter paper (Whatman #2). Collect the filtrate in a 4-ounce glass bottle which contains a layer of sodium sulfate about 0.5 cm deep. Seal the bottle with a Polyseal cap and retain the extract for analysis.

Orange molasses: Weigh a 30.0 gram subsample of the molasses directly into a 1-pint blender jar and allow it to thaw. Add 120 mL (or 104 g) of toluene with a graduated cylinder or laboratory-calibrated pipette and 30 mL of distilled water. Seal the top of the blender jar with aluminum foil, screw the cap on tightly, and blend the contents in the Waring blender at moderate speed for three minutes. An emulsion is formed. Pour out the contents into an 8-ounce wide-mouthed jar, seal the lid with aluminum foil and cap. Centrifuge the extract in the 8-ounce wide-mouth jar at 2000 rpm for 3 minutes. Pipette most of the top layer of clear toluene extract into a 4-ounce narrow-mouth bottle and desiccate by shaking it with a few grams of sodium sulfate. Clean-up procedures are given in section 2.3.4.

Dried orange pulp: Weigh a 30.0-gram subsample of the dried orange pulp directly into the Waring blender jar and allow it to thaw. Add 120 mL (or 104 g) of toluene and 120 mL of water just before blending to aid in moistening and macerating the dried pulp. Blend the contents for 3 minutes at medium speed. Scrape out the emulsified sample out of the blender jar into a wide-mouth 16-ounce jar, seal with aluminum foil, cap, and centrifuge it for 10 minutes at 2000 rpm. Pipette off the clear top layer toluene extract and deposit it into a 4-ounce narrow-mouth jar. At this step, the extract can be analyzed by GC-MSD. However, for GC-NP analysis, it is necessary to clean-up the remaining extract. Refer to section 2.3.4 for the details of the clean-up.

Orange oil: Weigh a 5.0-gram subsample of orange oil into a 60-mL separatory funnel. Pipet 40 mL of acetonitrile into the separatory funnel and shake the contents by hand for 3 minutes or until oil bubbles are no longer observed in the mixture. Clean-up procedures are given in section 2.3.4.

2.3.3 Napropamide fortification. Untreated control samples are analyzed to demonstrate freedom from interferences, and fortified control samples are analyzed to demonstrate method recovery. It is recommended that one unfortified and one fortified sample be analyzed for each commodity matrix for every set of ten (or fewer) samples extracted. Appropriate microliter syringes and volumes are used to fortify at the method's limit of quantitation (LOQ, 0.05 - 0.25 $\mu\text{g/g}$). For example, 30.0-g sample fortified at 0.05 $\mu\text{g/g}$ would require injection of 0.15 mL of a 10- $\mu\text{g/mL}$ fortification solution. Additional sample fortification at higher concentration levels may be needed depending on the range of residues.

2.3.4 Clean-up.

Molasses and dried pulp: Both extracts had the same column clean-up steps. Place 5.0-g alumina into each chromatography column and top with about 1 cm of dry, granular sodium sulfate. Pipette 20 mL of the toluene extract into the column. After the extract is drained though, add another 10 mL of reagent toluene, and discard these first eluates. Collect all the next eluates together in a 50-mL centrifuge tube. These will contain the napropamide residue. Add 30 mL of the 1:3 acetonitrile:toluene mixture, and then add 10 mL of 1:1 acetone:toluene. Place the centrifuge tube into the

Evapo-mix and evaporate the solvents slowly at room temperature with vacuum to avoid bumping to almost dryness (< 0.5 mL). Pipette enough reagent grade toluene into the centrifuge tube to equal 10 mL. Add approximately 2 g of anhydrous, granular sodium sulfate, cap the centrifuge tube with a glass or TFE stopper and vigorously shake it. A portion of this extract is directly injected onto the GC-NP or GC-MSD columns. (Although the dried orange pulp was not cleaned up prior to the GC-MSD analyses, it would be expected that it would give excellent quantitative results.)

Orange oil: The orange oil acetonitrile extract is cleaned up slightly differently. Wash the entire 40-mL acetonitrile extract with hexane twice. Add 15 mL of hexane to the separatory funnel containing the extract. Shake for at least 1 minute and allow it to separate for 10 minutes. Decant the bottom, acetonitrile layer into another separatory funnel and then add the second 15-mL wash of hexane, shake it for 1 minute, and separate the bottom layer into a 50-mL centrifuge tube. Place the 50-mL centrifuge tube into the Evapo-mix and evaporate the solvent slowly to less than 0.5-mL volume. Add 40 mL of toluene and shake the centrifuge tube to redissolve the residue.

The sample extract is now ready for column clean-up. Prepare a Pyrex column of the same dimensions as in the molasses and orange peel clean-up procedure, except that 8.00 grams of activated alumina is used. Pour the entire 40 mL of toluene extract into the top of the dry column. Discard this eluate. Then, pour 30 mL of the 1:3 acetonitrile:toluene solution into the column and collect the eluate into a 50 mL centrifuge tube. Pour another 10 mL of 1:1 acetone:toluene into the column and collect and combine the eluate into the same centrifuge tube.

Place the centrifuge tube into the Evapo-mix and slowly evaporate it to almost dryness (< 0.5 mL). Add 20 mL of toluene to the centrifuge tube to redissolve the residue. Add approximately 2 g of anhydrous, granular sodium sulfate, cap the centrifuge tube with a glass or TFE stopper and vigorously shake it. A portion of this extract is directly injected onto the GC-NP or GC-MSD columns.

2.3.5 Derivatization. No derivatization is necessary for the analysis of napropamide.

2.3.6 Safety considerations. Personnel untrained in the routine safe handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any first aid procedures can be found in the Material Safety Data Sheets accompanying the chemical or available from the chemical supplier. In general, always wear safety glasses with side shields, work in well ventilated areas, avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

Flammable solvents used are toluene, acetonitrile, hexane, acetone. Avoid breathing vapor and work in a well ventilated area. Avoid contact with the skin and clothing.

In case of dermal napropamide contamination, remove all contaminated clothing and wash the affected skin area with soap and water. Wash eyes with plenty of water after any accidental contact.

2.4 Instrumentation

2.4.1 Description of the instrumentation. The manufacturer's guidelines are followed for operation of the GC-NP and the GC-MSD. The extracts are analyzed with splitless injection, capillary gas chromatography and nitrogen-phosphorus detection. Confirmation, if necessary, is done using a capillary gas chromatograph with mass selective detection. All details of the analysis are recorded. The specific conditions listed below were used to generate the GC-NP and GC-MSD data presented in this report.

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	<u>GC-NP</u>	<u>GC-MSD</u>
Carrier Gas:	Helium	Helium
Column:	J&W DB-1301 fused silica capillary: 0.53 mm x 15 m x 1 μ m film thickness	Dried orange pulp: HP-1 fused silica capillary 12 m x 0.2 mm x 1 μ m film thickness. Other orange commodities: J & W DB-17 10 m x 0.2 mm x 0.3 μ m
Column Head Pressure:	1 psig	5 psig
Inlet Type:	splitless	splitless
Carrier Flow:	10.5 mL/min	NA
Data Acquisition:	HP laboratory automated system	Dried orange pulp: HP UNIX workstation; other: HP Chemstation
Injection Temperature:	240° C	240° - 260° C
Detection Temperature:	300° C	NA

2.4.2 Instrument operating conditions.

	<u>GC-NP</u>	<u>GC-MSD Dried Pulp</u>	<u>GC-MSD Molasses & Oil</u>	<u>GC-MSD Juice</u>
Initial Oven Temperature:	100° C	100° C	150° C	175° C
Initial Time:	0.01 min	0.01 min	0.01 min	0.01 min
Oven Temperature Program Rate:	30° C/min	30° C/min	14° C/min	9° C/min
Oven Final Temperature:	240° C	260° C	260° C	260° C
Final Time:	3 min	9 min	4 min	3.5 min
Splitless Valve: Off:	0.8 min	0.5 min	0.5 min	0.5 min
Total Run Time:	8.0 min	15.5 min	12.5 min	13.2 min
Napropamide Elution Time:	5.5 min	5.1 min	7.5 min	7.5 min

The GC-NP conditions were used for all of the extracts except for the orange oil extracts which needed slower programming. For those the GC-NP oven temperature program rate was 25° C/minute from 100° to 230° C with a bake out at 240° C and a total heated run time of 11 minutes.

Using the above conditions in the table for the GC-NP, the elution time of napropamide for the orange oil extract is 6.37 minutes. Operation of the GC-MSD was in the selective ion monitoring mode (SIM) at 271 m/z. For optimum sensitivity, the GC-MSD must be manually tuned on the ions 219, 264, and 414 (see Fig. 9). See Figures 1 - 8 for typical chromatograms.

2.4.3 Calibration. Quantitation is performed using an external standard method. The GC-NP and GC-MSD are calibrated by using the calibration standards described in section 2.4. The 0.1, 0.05, 0.025 and 0.01 µg/mL solutions are injected at the beginning and at the end of each run to assure linearity. After every four or five samples, one or more of the calibration solutions is analyzed to assure that the analyte response is stable within 10 %. If no residues are detected and only the lower LOQ method fortifications are used, only the 0.01 µg/mL calibration solution and another standard solution is needed.

The sample extracts are injected using the same conditions as those for the calibration solutions. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of the retention time (\pm 0.05 min) with that of the analyte peak in the calibration solution chromatograms.

2.5 Interferences

GC-NP and GC-MSD analyses of untreated sample extracts demonstrate the presence or absence of significant interferences from sample matrices, solvents, and labware.

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GC-NP extracts of the untreated, unfortified samples of orange oil showed slight ($<0.05 \mu\text{g/g}$) co-extractive impurities that interfered with analyte peaks. These interferences were confirmed to be the ion similar to the parent of napropamide by analyses using the GC-MSD. There was one slight GC-NP interference found in the reagent blank sample for the orange juice set which was considered an anomaly.

Also, interferences ($<0.05 \mu\text{g/g}$) were found in the GC-MSD analyses of dried orange pulp, orange oil, and orange juice.

Figures 1-4 show GC-NP typical chromatograms of respective calibration solutions of napropamide, untreated control extracts, and 0.05-ppm fortified sample extracts for each commodity.

Figures 5-8 show GC-MSD typical chromatograms of respective calibration solutions of napropamide, untreated control extracts, and 0.05-ppm fortified sample extracts for each commodity.

2.6 Confirmatory Techniques

Residues of napropamide in untreated controls or treated samples above the lower LOQ ($0.05 \mu\text{g/g}$) should be confirmed by using capillary GC-MSD with a selective ion monitoring at m/z 271. Selecting alternative mass ion(s), such as m/z 100, m/z 128, and m/z 171 (see Fig. 9c) and/or using a GC column of different polarity, are other possible techniques of confirmation of napropamide residues. Quantitation is performed using the external standard method.

2.7 Time Required for Analysis

The extraction of up to six samples for a specific commodity matrix can be completed by one person in an 8-hour period, provided that samples of orange juice, molasses, dried pulp, and oil are available, subsampled, and thawed for use. The instrument time needed for analysis is 10 - 20 minutes between each injection, which therefore requires a 3 to 4-hour run with the injection of calibration standards.

2.8 Modifications or Potential Problems

No potential problems were observed during GC-NP method validation for the orange juice. However, orange molasses and dried pulp required activated alumina column clean-up and the orange oil required solvent washes and activated alumina column clean-up.

The orange oil chromatograms had some interferences which required different GC-NP oven temperature programming than the other matrices. Likewise, the GC-MSD required different oven temperature programming for some orange matrices. (refer to sections 2.4.1 and 2.4.2.

2.9 Calculations

2.9.1 Response factor determination. The concentration of the analyte in the matrix is calculated by an appropriate external standard method; i.e., the response obtained for the analyte in the sample extract is compared to the response obtained from a separate injection of a known amount of analyte (calibration solution). In order to use the linear response calculation method shown below, the injection volumes for all calibration solutions and sample extracts are fixed at the same volume.

The response factor, F , is calculated from an injection of a calibration solution as follows:

$$F = \frac{C_{std}}{R_{std}}$$

where C_{std} = concentration of calibration solution, $\mu\text{g/mL}$

R_{std} = response units (e.g., peak height, peak area) from detector for the calibration solutions

2.9.2 Analyte, napropamide in sample. Determine the concentration of napropamide in the commodity sample, C , using the average response factor as follows:

$$C = R_{sample} \times [F_{avg} / T]$$

C = concentration ($\mu\text{g/g}$, ppm)

where R_{sample} = response units (e.g., peak heights or peak area) from detector for sample extract

F_{avg} = average response factor over the range, [$(\mu\text{g/mL})/\text{response unit}$]

T = commodity/solvent ration (g commodity/mL solvent) in the injected solution

The quantity, [F_{avg} / T], is also known as the sample calculation factor ($\mu\text{g/g}$ response units) which is different than the response factor ($\mu\text{g/mL}$ response units).

2.10 Other: Matrix Effects Test and Extractability Study

2.10.1 Matrix effects test. Matrix effects are defined as any enhancement or reduction of instrument detector response for napropamide in the presence of commodity extractives, when compared to the response in pure calibration solutions.

A simple procedure was developed to test for such matrix effects. Aliquots of untreated sample extracts were fortified with known amounts of napropamide prior to analysis: 10 mL aliquots were fortified by the injection of 0.025 mL of the 10 $\mu\text{g/mL}$ -calibration solution into the GC-prepared samples. Recoveries of these matrix fortifications and the method fortifications were compared by using the same instrument operating conditions as the method.

3 RESULTS/DISCUSSION

3.1 Accuracy of the Residue Method

A set of six fortified samples was prepared for each processed orange matrix as described under section 2.3.3. These samples were extracted and analyzed to establish accuracy of the residue method. Tables 1 - 4 list the napropamide recoveries for each proposed orange matrix.

The gas chromatography method showed that recoveries of napropamide from orange juice fortified from 0.05 to 0.25 $\mu\text{g/g}$ ranged from 95 % to 109 % with a mean recovery of 100 %. See Figure 1 on page 35 for example chromatograms.

Recoveries of napropamide from dried orange pulp fortified from 0.05 to 0.25 $\mu\text{g/g}$ ranged from 93 % to 115 % with a mean recovery of 101 %. See Figure 2 on page 36 for example chromatogram.

Recoveries of napropamide from orange molasses fortified from 0.05 to 0.25 $\mu\text{g/g}$ ranged from 93 % to 99 % with a mean recovery of 95 %. See Figure 3 on page 37 for example chromatogram.

Recoveries of napropamide from orange oil fortified from 0.05 to 0.25 $\mu\text{g/g}$ ranged from 80 % to 100 % with a mean recovery of 96 %. See Figure 4 on page 38 for example chromatogram.

Table 4 lists the results of the analyses using gas chromatography with mass selective detection. Recoveries of napropamide in orange juice, dried orange pulp, orange molasses and orange oil fortified at 0.05 $\mu\text{g/g}$ were 71 %, 79 %, 101 % and 105 %, respectively. Dried orange pulp fortified at 0.25 $\mu\text{g/g}$ averaged 86 % recovery. See Figures 5 - 9 for example GC-MSD chromatograms.

3.2 Precision

The precision of a method depends on variations in extraction, clean-up, and instrumental analysis. These variations can be evaluated from the recovery data obtained in extraction and instrumental analyses can be evaluated during analyses of fortified samples.

The coefficients of variation, CV, (% relative standard deviation) of napropamide recovery have been calculated for each sample commodity, based on the 0.05 and 0.25 fortifications. This data is presented in Tables 1 - 5 for orange juice, dried pulp, molasses, and oil. For each respective orange extract the CV is 5.3 %, 7.9 %, 2.2 %, and 8.2 %. The CVs were calculated from the percent recoveries on the GC-NP instrument.

Instrumental precision for GC-NP analyses of orange juice, dried pulp, molasses, and oil is determined by the CV of each set of peak heights of calibration standards at 0.025 µg/mL in each run. The instrumental precision for each GC-NP run for the orange juice, pulp, molasses, and oil are 2.8 %, 3.8 %, 4.7 %, and 8.7 %.

The GC-MSD had less instrumental precision than the GC-NP. For the orange dried pulp matrix, the CV of the 0.025-µg/mL peak heights was 5.9 %. For the orange molasses and oil matrices, the CV of the 0.025-µg/mL peak heights was 6.0 %, respectively. For the orange juice matrix, the CV of the 0.025-µg/mL peak heights was 8.2 %.

3.3 Limits of Quantitation

The limit of quantitation for the GC-NP and GC-MSD method is 0.05 µg/g for napropamide, as determined by fortifications at the 0.05-µg/g level. With the sample weight and volume of extract specified as 1 g / 4 mL, this value is equivalent to 0.0125 µg/mL of injected solution. Adequate sensitivity was achieved at this concentration level, with peak heights above 1 cm for napropamide.

3.4 Ruggedness Testing

The system was not tested for ruggedness.

3.5 Limitations

In this study, the level of quantitation was between 0.05 and 0.25 µg/g (ppm). If napropamide residues exceed 0.25 µg/g, the method must be further validated by analyses of untreated control samples fortified at a concentration which matches or exceeds the levels expected in the treated crop sample.

3.6 Matrix Effects

Results of matrix effects testing are given in Tables 6 and 7. There was no significant increase or decrease in the nitrogen-phosphorus detector response to napropamide in this test on orange juice, orange molasses, or orange oil. Matrix effects in dried orange pulp were not tested using the GC-NP.

A positive matrix effect was seen in the confirmatory GC-MSD analyses for orange juice. This analysis was considered unusual because whole chopped oranges and all the other orange commodities did not show any enhanced responses. The apparent matrix effect for orange juice may be related to the choice of GC column for the analysis. In any future confirmatory analysis for napropamide residues in orange juice, an additional matrix test sample should be prepared and analyzed concurrently with the juice extracts. If an enhancement of peak height response is observed, it should be further verified by using a GC column of different polarity.

3.7 Extractability Results

There are no extractability results because no radioactive residues were found in the metabolism tree crop study with which to compare with this residue method.

The extractability of napropamide was previously validated on crops using ^{14}C , radiolabelled material (ref. 8.4).

4 CONCLUSION

A method for the analysis of napropamide in processed commodities of oranges at levels of 0.05 $\mu\text{g/g}$ to 0.25 $\mu\text{g/g}$, has been validated. A confirmatory technique was performed using gas chromatography with mass selective detection.

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The method is specific for the analysis of napropamide in dried orange pulp, orange juice, orange molasses, and orange oil. Only commercially available laboratory equipment and reagents are required. The analysis can be completed for each matrix by one person in an 8 hour period if the samples are prepared. Untreated and fortified untreated samples should be extracted and analyzed with each set of treated samples to demonstrate absence of interferences and adequate recovery. If determination of napropamide at a concentration other than 0.05 $\mu\text{g/g}$ to 0.25 $\mu\text{g/g}$ is required, suitably fortified samples must be analyzed to validate the method at that concentration.

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Figure 7. Example Chromatograms for the Analysis of Orange Molasses Using GC-MSD Chromatography

Figure 8. Example Chromatograms for the Analysis of Orange Oil Using GC-MSD Chromatography

Figure 9. GC-MSD Chromatograph of Napropamide

Table 1. Recovery of Napropamide from Orange Juice Using GC-NP

<u>Sample Number</u>	<u>Weight Crop (g)</u>	<u>Amount Added ($\mu\text{g/g}$)</u>	<u>Response (height)</u>	<u>Recovery^a (%)</u>	<u>Amount Found ($\mu\text{g/g}$)</u>
G3046-01	0	0	184		<0.05
G3046-02	30.0	0	0		<0.05
G3046-03	30.0	0	26		<0.05
G3046-04	30.0	0.05	2654	109.3	0.058
G3046-05	30.0	0.05	2333	95.4	0.051
G3046-06	30.0	0.25	2483	101.9	0.054
G3046-07	30.0	0.25	11203	96.0	0.243
G3046-08	30.0	0.25	11430	98.0	0.248
G3046-09	30.0	0.25	11341	97.2	0.246

Total Number of Samples Analyzed: 6

Sample Mean Recovery: 99.6 %

Sample Coefficient of Variation: 5.3 %

Instrumental CV: 2.8 %

^a Recoveries are corrected for average peak height response found in the untreated controls.

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Table 2. Recovery of Napropamide from Dried Orange Pulp Using GC-NP

<u>Sample Number</u>	<u>Weight Crop (g)</u>	<u>Amount Added ($\mu\text{g/g}$)</u>	<u>Response (height)</u>	<u>Recovery^a (%)</u>	<u>Amount Found ($\mu\text{g/g}$)</u>
G3043-01	0	0	0		<0.05
G3043-02	30.0	0	172		<0.05
G3043-03	30.0	0	177		<0.05
G3043-04	30.0	0.05	1924	93.3	0.051
G3043-05	30.0	0.05	2011	97.9	0.054
G3043-06	30.0	0.25	2039	99.4	0.054
G3043-07	30.0	0.25	9027	94.4	0.241
G3043-08	30.0	0.25	10926	114.6	0.291
G3043-09	30.0	0.25	9992	104.7	0.266

Total Number of Samples Analyzed: 6
Mean Recovery 100.7 %
Sample Coefficient of Variation: 7.86 %
Instrumental CV 4.7 %

^a Recoveries are corrected for average peak height response found in the untreated controls.

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Table 3. Recovery of Napropamide from Orange Molasses Using GC-NP

<u>Sample Number</u>	<u>Weight Crop (g)</u>	<u>Amount Added ($\mu\text{g/g}$)</u>	<u>Response (height)</u>	<u>Recovery^a (%)</u>	<u>Amount Found ($\mu\text{g/g}$)</u>
G3044-01	0	0	0		<0.05
G3044-02	30.0	0	0		<0.05
G3044-03	30.0	0	118		<0.05
G3044-04	30.0	0.05	1998	98.8	0.051
G3044-05	30.0	0.05	1954	96.6	0.050
G3044-06	30.0	0.25	1881	92.8	0.048
G3044-07	30.0	0.25	9296	94.1	0.237
G3044-08	30.0	0.25	9361	94.8	0.239
G3044-09	30.0	0.25	9374	94.9	0.239

Total Number of Samples Analyzed: 6

Mean Recovery: 95.3 %

Sample Coefficient of Variation: 2.20 %

Instrumental CV: 3.8 %

^a Recoveries are corrected for average peak height response found in the untreated controls.

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Table 4. Recovery of Napropamide from Orange Oil Using GC-NP

<u>Sample Number</u>	<u>Weight Crop (g)</u>	<u>Amount Added ($\mu\text{g/g}$)</u>	<u>Response (height)</u>	<u>Recovery^a (%)</u>	<u>Amount Found ($\mu\text{g/g}$)</u>
G3045-01	0	0	0		<0.05
G3045-02	30.0	0	742		<0.05
G3045-03	30.0	0	780		<0.05
G3045-04	30.0	0.05	2300	100.3	0.075
G3045-05	30.0	0.05	2214	94.7	0.072
G3045-06	30.0	0.25	1994	80.3	0.065
G3045-07	30.0	0.25	7686	100.1	0.275
G3045-08	30.0	0.25	7698	100.3	0.276
G3045-09	30.0	0.25	7550	98.2	0.270

Total Number of Samples Analyzed: 6

Mean Recovery: 95.7 %

Sample Coefficient of Variation: 8.2 %

Instrumental CV: 8.6 %

^a Recovery are determined by subtracting the responses found in the untreated control.

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Table 5. Recovery of Napropamide from Orange Juice, Dried Orange Pulp, Orange Molasses and Orange Oil Using GC-MSD

Sample Number	Weight Crop (g)	Amount Added ($\mu\text{g/g}$)	Response (height)	Recovery ^a (%)	Amount Found ($\mu\text{g/g}$)
Orange Juice:					
G3046-02	30.0	0	71 ^b		<0.050 ^b
G3046-04	30.0	0.05	560	70.5	0.040
Dried Orange Pulp:					
G3043-02,03	30.0	0	3132 ^b		<0.050 ^b
G3043-04,05,06	30.0	0.05	23108 ^b	79.1 ^b	0.046 ^b
G403-07,08,09	30.0	0.125	12486 ^b	86.2 ^b	0.222 ^b
Orange Molasses:					
G3044-02	30.0	0	0		<0.05
G3044-04	30.0	0.05	1420	101.2	0.051
Orange Oil:					
G3045-02	30.0	0	1020 ^b		<0.05 ^b
G3045-04	30.0	0.05	2494	105.0	0.089

Instrumental CV:

Pulp: 5.9 %
 Juice: 8.3 %
 Molasses and Oil: 6.0 %

^a Recoveries are corrected for average peak height response found in the untreated controls.

^b Average of replicate injections.

Table 6. Recovery of Napropamide from Orange Juice, Dried Orange Pulp, Orange Molasses, and Orange Oil Matrix Fortifications Using GC-NP

Sample Number	Amount Added ($\mu\text{g/mL}$)	Response (height)	Recovery ^a (%)	Amount Found ($\mu\text{g/mL}$)
Orange Juice:				
G3046-02	0	0		<0.012
G3046-10	0.025	4509	97.8	0.034
Dried Orange Pulp:				
G3043-02	NA ^b	NA	NA	NA
G3043-10	NA	NA	NA	NA
Orange Molasses:				
G3044-02	0	0		<0.012
G3044-10	0.025	3791	96.6	0.024
Orange Oil:				
G3045-03	0	780		<0.012
G3045-10	0.025	3792	98.2	0.031

^a Recovery are corrected for the peak height response found in the untreated controls.

^b Not analyzed.

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Table 7. GC-MSD Recovery of Napropamide from Orange Juice, Dried Orange Pulp, Orange Molasses, and Orange Oil Matrix Fortifications

<u>Sample Number</u>	<u>Amount Added ($\mu\text{g/mL}$)</u>	<u>Response (height)</u>	<u>Recovery^a (%)</u>	<u>Amount Found ($\mu\text{g/mL}$)</u>
Orange Juice:				
G3046-02	0	71		<0.012
G3046-10	0.025	1909.4	130.8	0.025
Dried Orange Pulp:				
G3043-02	0	667		<0.012
G3043-10	0.025	50774	99.1	0.025
Orange Molasses:				
G3044-02	0	0		<0.012
G3044-10	0.025	3115.4	111.0	0.031
Orange Oil:				
G3045-02	0	120.3		<0.012
G3045-10	0.025	4096.4	109.6	0.036

^a Recoveries are corrected for average peak height response found in the untreated controls.