Removal of Pesticides From Citrus Oils Using RENSA® 101

This application note demonstrates the efficient and selective removal of a wide range of pesticides from natural lemon oil using RENSA® 101.

Abstract

A novel designed adsorbent, RENSA® 101 is presented that is able to clean-up citrus flavor oils from undesired pesticide residues. The selective adsorbent enables the targeted and quantitative removal of a range of pesticides from citrus flavor oils without altering their overall composition. All vital components that are characteristic for the flavor and taste are largely unaffected by the clean-up process.

Introduction

Pesticides are widely used in the agricultural business to protect fruits and other plants from the attack of insects, molds and other pests. Although new generations of pesticides are both effective and have good biodegradability a large range of pesticides may persist as chemical residues in products originating from e.g. fruits. One such example is the presence of minute quantities of pesticides in citrus oils extracted from the peels of citrus fruits. There is a wide range of flavor oils available from citrus fruits such as lemon, orange, lime, bergamot and others. Citrus oils are important ingredients in a large variety of food, fragrance and beverage products.

Traditional clean-up methods utilizing standard unit operations such as distillation are not effective in removing such pesticides without negatively affecting essential organoleptic properties and characteristics of citrus flavor oils. In the social media, the presence of minute amounts of pesticides in fruit soft drinks has caused broad public attention towards this issue (1).

MIP Technologies AB (a subsidiary of Biotage AB) has developed the designed resin RENSA® 101 that features built-in engineered binding site cavities. These binding site cavities are highly selective and recognize a large number of different organophosphorus pesticides (and some other classes of pesticides) in citrus oils and other hydrophobic oils and matrices.

Here we demonstrate the quantitative and selective removal of eight pesticides (structures shown in Figure 1) from spiked lemon oil by passing the spiked oil through a column packed with RENSA® 101. Using this selective resin, the pesticide contamination levels in lemon oil are strongly reduced while at the same time its desirable flavor and aroma properties are retained.

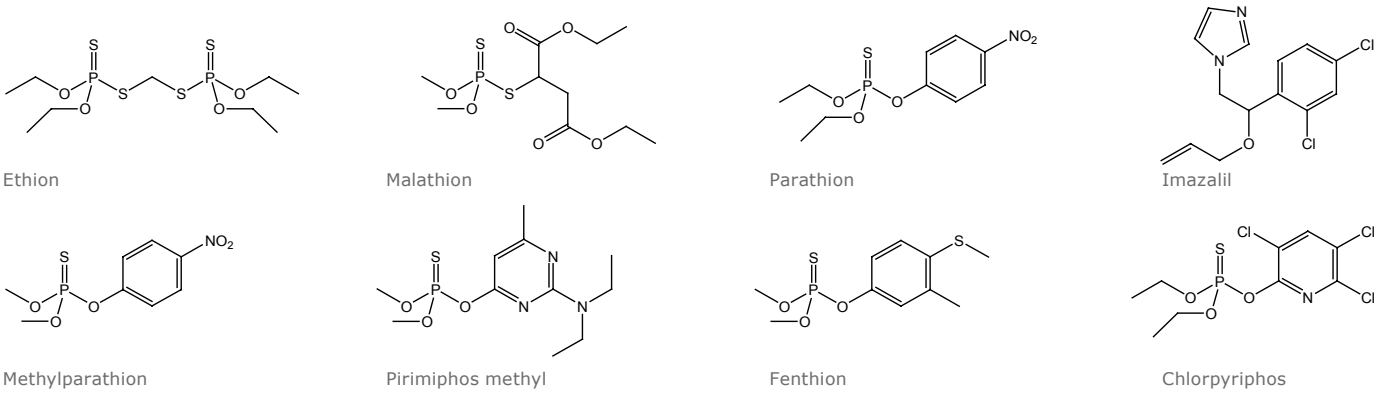

Figure 1. Chemical structures of the pesticides tested. Pesticides shown are seven organophosphorus insecticides and imazalil which is an imidazole fungicide.

Natural lemon oil is a complex mixture containing many different compounds. As the main constituents of citrus oils are limonene and other terpene compounds citrus oil therefore presents a strongly hydrophobic environment. The chemical structures of some of the important lemon oil constituents are shown in Figure 2.

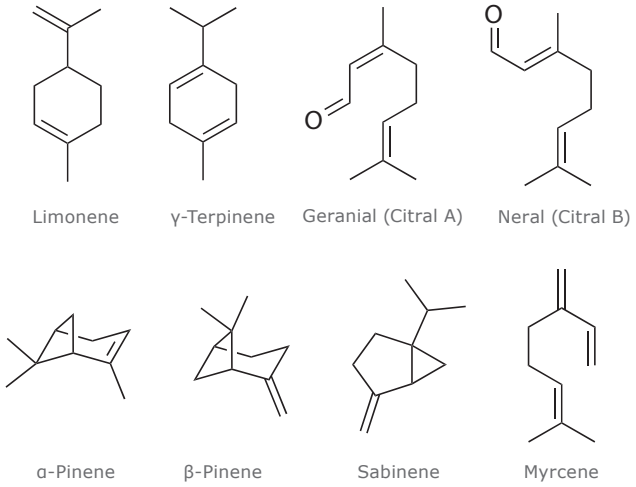

Figure 2. Typical constituents of natural lemon oil.

In addition to the terpenes lemon oil contains oxygenated derivatives such as neral or geranial and a multitude of alcohols, aldehydes, esters, unsaturated hydrocarbons and also non-volatile constituents including waxes and colored pigments.

Other citrus oils are similar in their overall composition, but will contain different characteristic flavor and aroma compounds and may also differ in the relative amounts of the typical citrus oil components giving them their unique fragrance and flavor properties.

Column Purification Using RENSA® 101

Citrus oils can be treated with RENSA® 101 in a column format, where the polymeric adsorbent is packed as a bed in a column. The citrus oil is passed through the packed bed and the undesired toxic pesticides will be strongly retained while the citrus oil constituents pass through. Before starting the clean-up process the column is pretreated as a first step. Step 1 is a conditioning step where the resin is allowed to swell in an appropriate solvent. In step 2, the process solution is then allowed to pass through the column. Typically, it is recommended to discard the first 2 column volumes of the process solution. Usually, the total amount of process solution that can be treated is determined by the binding capacity of the column and the column can be operated until it is saturated.

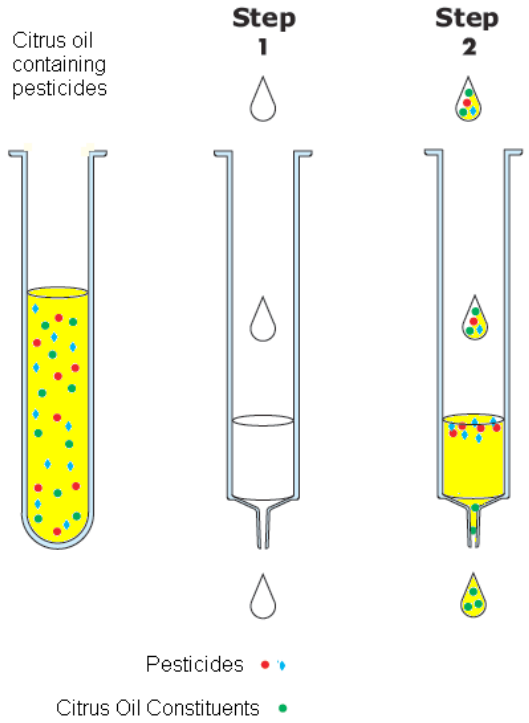

Figure 3. RENSA® purification principle.

Experimental

Materials

Lemon oil and limonene (R)-(+) were purchased from Sigma-Aldrich. RENSA® 101 is from MIP Technologies AB (a subsidiary of Biotage AB), Lund, Sweden. Pesticides (ethion, malathion, parathion, imazalil, fenthion, chlorpyriphos, methylparathion and pirimiphos methyl) were of analytical grade and purchased from Sigma-Aldrich. Pyrene was purchased from Supelco and used as internal standard in the binding capacity measurements. Empty ISOLUTE® polypropylene SPE columns were from Biotage AB, Uppsala, Sweden.

Analysis

Analyses of the various samples were carriet out on a 6890N/5975 GC-MS system from Agilent Technologies. Chromatographic separation was performed on a fused silica capillary column, DB-5MS (30 m length, 0.25 mm i.d., 0.25 µm film thickness) from J&W Scientific. Helium was used as a carrier gas at a constant flow of 1.2 mL/min. The column temperature was programmed from 70 °C to 175 °C at the rate of 50 °C/min, increased to 230 °C at the rate of 9 °C/min and finally to 280 °C at the rate of 16 °C/min. The final temperature was held for 4.0 minutes. Total run time was 15.3 min. 2 µL of the sample was injected in split-less mode. Quantitative analysis was carried out by measuring the qualifier ions m/z

231 for ethion, m/z 173 for malathion, m/z 291 for parathion and m/z 202 for pyrene.

Lemon Oil Clean-Up Procedure

Isolute SPE columns (3 mL size) were packed with 400 mg of RENSA® 101. 1 mL of clean lemon oil was added to the column and allowed to swell for 1 hour. The material swells to approximately 3 times its initial bed volume. Then 1 mL of a lemon oil sample, spiked with 250 ppm of each of the 8 different pesticides, was added and allowed to slowly pass through the packed column bed of RENSA® 101. The spiked lemon oil samples were analyzed on the GC/MS before and after clean-up. As a comparison, a natural (non-spiked) lemon oil sample was also analyzed by GC/MS. All experiments were conducted at ambient temperature.

Table 1. Lemon oil clean-up procedure.

Column configuration	ISOLUTE column 3ml, packed with 400 mg of RENSA 101
Column pre-treatment	Load 1 mL of neat lemon oil
Resin swelling	Let resin swell in column for 1 hour. Resin will swell by at least 120 %.
Lemon oil clean-up	Load spiked lemon oil Collect cleaned-up lemon oil

Binding Tests

Neat limonene and natural lemon oil were used to determine the effect of the matrix on the resin capacity. Binding capacity tests were done in flow mode and in batch mode to study the effect of different contact times.

In flow mode ISOLUTE® 10 mL SPE columns were filled with 200 mg of RENSA® 101. 1 mL of limonene (or lemon oil) was added to the column and the resin was allowed to swell for 1 hour. In order to determine the binding capacity, break-through curves were established. The maximum binding capacity was defined at the point where 1 % pesticide break-through occurred. Twenty times 1 mL of samples containing a pesticide in either limonene or lemon oil were added.

In batch mode, 50 mg of RENSA® 101 was added to 20 mL vials and 10 mL of pesticide spiked limonene was added to each vial. Samples of 50 µL were withdrawn after 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours and analyzed on the GC-MS system after dilution with 250 µL limonene.

Results and Discussion

The capability of RENSA® 101 to efficiently and selectively remove a range of pesticides from lemon oil is illustrated in Figure 4. The chromatograms of spiked lemon oil and spiked lemon oil after clean-up using RENSA® 101 are shown. As a comparison, a chromatogram of non-spiked lemon oil is also included.

In Figure 4 it can be seen that the chromatograms of non-spiked and cleaned-up lemon oil are virtually identical. All pesticide peaks are quantitatively removed by the use of RENSA® 101 (analytical quantitative data not shown). In addition, important volatile constituents that elute early on (up to 5 minutes elution time) are not affected by the treatment.

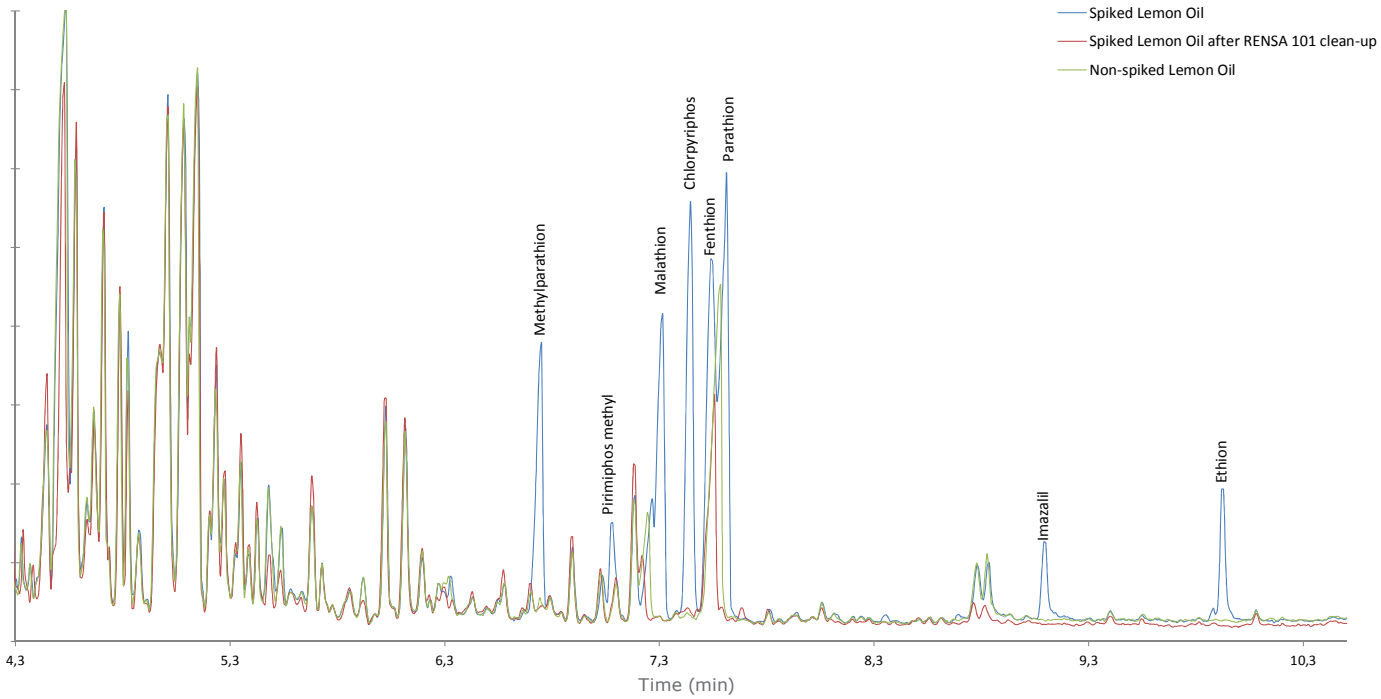

Figure 4. View of the total ion scan GC-MS chromatograms of the various samples of lemon oil.

As can be seen in Table 2, the binding capacity of RENSA® 101 is influenced by the environment in which the pesticides are present. Neat limonene contains no competing compounds and thus provides a higher number of available binding sites for the pesticides. On the other hand, lemon oil contains a multitude of other compounds that may form weak interactions with the resin surface and influence the binding capacity.

Table 2. The binding capacity of the pesticides depends on the matrix. The binding capacity is given as mg pesticide/g RENSA® 101. These were tested in flow mode at 0.1 mL/min.

Matrix	Ethion	Malathion	Parathion
Limonene	59	54	12
Lemon oil	37	7	3

The binding capacity can be improved if required by increasing the contact time as the selective binding is time dependent. Table 3 demonstrates that in column mode, a slow column flow or a long contact time in batch mode, lead to higher binding capacity values.

Table 3. The binding capacity of RENSA® 101 in limonene depends on the contact time. The binding capacity is measured as mg pesticide/g RENSA® 101.

Contact time	Ethion	Malathion	Parathion
Flow mode: 1 mL/min	13	17	2
Flow mode: 0.1 mL/min	59	54	12
Batch mode: 24 h	59	57	43

Conclusions

RENSA® 101 is able to completely remove a wide range of pesticides from natural lemon oil without removing other vital constituents of lemon oil, as measured by analytical GC/MS measurements. The selective and quantitative removal of those pesticides from spiked lemon oil is accomplished at ambient temperature by a simple clean-up step, where the spiked oil is passing through a column packed with RENSA® 101. Both flavor and taste profiles are kept intact after treatment with RENSA® 101. Professional sensory panels have confirmed the integrity of citrus oils that have been cleaned up with RENSA® 101.

The binding capacity of the resin is at around 50 mg pesticide/g resin under ideal conditions. At typical contamination levels that can be found in actual lemon oil products (~ 0.5 ppm), 1 gram of RENSA® 101 may be sufficient to clean up about 5 liters of contaminated citrus oil, which allows for an economical and technically feasible process to be implemented.

References

1. www.sciencedaily.com, Dec. 16, 2008.

Ordering Information

Part Number	Description	Quantity
95002-0001	RENSA® 101	1 g
95002-1000	RENSA® 101	1 kg
----	Industrial scale quantities are available on request	Please inquire

Safety Information

Material Safety Data Sheets (MSDS) are available for all MIP Technologies resin products and we recommend that you obtain copies of our MSDS from MIP Technologies technical services or visit our homepage www.biotage.com before using our products in your facilities.

MIP Technologies strives to deliver polymeric resins at the best quality and consistency. Depending on the polymer resin product and its production process, it may contain residuals or by-products originating from the manufacturing process. The user is advised to determine the levels of the residuals and to which extent they may need to be removed for their particular use. MIP Technologies may also provide polymeric resins of alternative purity grades upon request. Consult your MIP Technologies technical services for further information. All our products are produced in ISO 9001 certified manufacturing facilities.

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