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**Colour Tests for Precursor Chemicals of Amphetamine-Type  
Substances**

**Systematic study of colour tests for safrole and safrole-rich essential oils**

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## INTRODUCTION

### Abstract

Safrole (3,4-methylenedioxyallylbenzene) is a precursor chemical in the clandestine synthesis of several ring-substituted amphetamine-type substances, such as 3,4-methylenedioxy-N-methylamphetamine (MDMA, “ecstasy”), 3,4-methylenedioxyamphetamine (MDA), N-hydroxy-3,4-methylenedioxyamphetamine (N-Hydroxy-MDA) and 3,4-methylenedioxy-N-ethylamphetamine (MDE). In recent years, there has been concern about the use of safrole-rich oils in illicit MDMA manufacture.

The present paper describes a systematic study on different colour tests for safrole and safrole-rich essential oils. The study tested various colour reagents for their potential to:

- (I) identify safrole, whether pure or in the presence of other - potentially interfering - essential oil components, and
- (II) distinguish, on a semi-quantitative basis, essential oils of high safrole content from those of low content.

The study was aimed at using, to the extent possible, colour tests already included in the UNODC precursor field identification kit, and to propose simple modifications to the existing tests and procedures, where required. It was found that a combination of the Marquis test, the gallic acid test, concentrated sulfuric acid and the phloroglucinol reagent allows to differentiate between safrole-rich essential oils and samples containing little or no safrole.

The following paper describes in detail the analytical approach of the study, including the findings in each step of the research protocol. It also provides relevant background information.

The proposed operating procedure for the field identification of safrole-containing essential oils is provided in annex 1.

## Background

### Why focussing on safrole-rich oils?

Safrole is commercially available for legitimate purposes (e.g., production of heliotropine for fragrance and flavouring applications or the production of piperonyl butoxide (PBO) for use as a pyrethrum synergist). Safrole can be synthesized from basic and uncontrolled chemicals. However, it is also abundantly available from natural sources.

Common natural sources of safrole include different species from the *Lauraceae* (e.g., *Sassafras albidum*, *Cinnamomum camphora*, *Ocotea cymbarum* and *Ocotea pretiosa*) and *Piperaceae* (e.g., *Piper hispidinervium*) plant family. Safrole-containing plants are found in North America, South America, East Asia and South East Asia. [1]



Figure 1: *Cinnamomum camphora*

Safrole-rich essential oils are usually obtained by steam distillation of wood, rootstumps, branches and other parts of safrole-containing species.

Safrole can be present in natural essential oils at concentration levels ranging from a few percentages to more than 90% safrole. With such high concentrations of safrole, some oils can virtually be considered as pure safrole.



Figure 2: *Cinnamomum camphora*, leaves

Safrole-rich oils are the main raw materials for the manufacture of safrole for commercial purposes. The oils are also used in traditional medicine. The use of safrole and safrole-rich oils in food and consumable products has been banned internationally.

An extensive survey on the production, trade and use of essential oils rich in safrole has recently been completed in East and South East Asia. [2]

### **Project Prism: International action against Precursors Required In Synthetics Manufacture**

Safrole is controlled as an essential precursor in Table 1 of the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances (aka 1988 Convention). In addition, to respond to the need for coordinated action at the global level, a voluntary international project (“Project Prism”) was launched in 2002, aimed at assisting governments in developing effective mechanisms for the monitoring and control of ATS precursors, to prevent their diversions and carry out follow-up investigations to identify their sources.

In response to a resolution by the Commission on Narcotic Drugs (CND) in 2006, the International Narcotics Control Board (INCB) has recently adopted the following definition for safrole-rich oils [3]:

“safrole/safrole-rich oils are any mixtures or natural products containing safrole present in such a way that it can be used or recovered by readily applicable means”.

#### **Why field tests for identifying safrole and safrole-rich oils?**

#### **Why the need for semi-quantitative determination of the safrole content of safrole-rich oils?**

Safrole and its side-chain isomer isosafrole can be detected by colour tests, allowing the rapid presumptive field identification. Such tests are required, for example, when police and customs personnel, often in remote locations, need rapid information on whether a suspected material is a controlled drug or precursor or not. Whenever presumptive positive results are obtained using a field identification kit, the suspected material must be submitted to an authorized laboratory for further confirmatory analyses.

Considering that oils with low safrole concentrations, or from which safrole cannot be easily obtained (see definition above), are less attractive for illicit operators, it was one of the aims of this study to determine the possibility of an on-the-spot (semi-quantitative) differentiation of oils based on their safrole content.

## Objectives

The aim of this study therefore was to systematically test and document the results of safrole and a number of safrole-rich oils, using colour tests contained in the United Nations Precursor Field Identification Kit and other suitable colour tests. Specifically, this included studies on:

- (I) whether all available oils (covering a range of levels of safrole and accompanying other ingredients) tested positive, and
- (II) the minimum safrole concentration that gave a positive colour test result.

Where required, work also included modifications of existing colour tests to improve their suitability, practicality and the stability of reagents.

## Procedure

1. GC-MS analysis of various samples of safrole-containing essential oils and estimation of their safrole content by GC-MS peak area percentage (for GC-MS operating conditions see annex 2).
2. Identification (by GC-MS and co-injection) of substances other than safrole which are present in natural oils at different levels and estimation of the content of these substances by GC-MS peak area percentage.
3. Testing reference samples (safrole and essential oil compounds that have been identified by GC-MS) with all available colour test reagents, including dilution studies to determine the safrole cut-off concentration below which colour tests do not consistently produce positive results.
4. Testing samples of safrole-containing essential oils with all available colour test reagents.
5. The results of the accomplished analyses were used to identify the best combination of reagents for the detection and semi-quantitation of safrole in essential oils.

## EXPERIMENTAL

### 1. Materials and Methods

All essential oil samples (from Cambodia, PR China, Indonesia, Lao PDR, Myanmar and Vietnam) and reference substances were provided by the Laboratory and Scientific Section of the United Nations Office on Drugs and Crime, Vienna, Austria.

#### 1.1. Essential oils tested

The essential oils tested were those in Table 1 below.

Table 1: List of essential oils tested

Country of origin	Sample code	Safrole (%)*	Other compounds*
Cambodia	UNX 1791	99	-
Unknown (seized in Germany)	UNX 1825	100	-

Country of origin	Sample code	Safrole (%)*	Other compounds*
PR China	UNX 1826a	92	-
PR China	UNX 1826b	92	-
PR China	UNX 1826c	91	-
PR China	UNX 1827a	97	-
PR China	UNX 1827b	98	-
PR China	UNX 1827c	98	-
PR China	UNX 1829	91	1,8-Cineole (Eucalyptol) 3% Camphor 2%
PR China	UNX 1902a	15	Camphor 42% 1,8-Cineole (Eucalyptol) 21% 1- $\alpha$ -Terpineol 5%
PR China	UNX 1902b	92	Asarone 3%
PR China	UNX 1902c	0	$\alpha$ -Cedrol 9%
PR China	UNX 1902d	84	Benzaldehyde 6% $\alpha$ -Terpinene 4%
PR China	UNX 1902e	42	Camphor 4% 1,8-Cineole (Eucalyptol) 18% $\alpha$ -Pinene 10% 2- $\beta$ -Pinene 21%
PR China	UNX 1902f	91	-
PR China	UNX 1902g	96	Limonene 3%
PR China	UNX 1902h	97	-
PR China	'Sassafras oil (Chinese)'	87	Camphor 3% 1,8-Cineole (Eucalyptol) 5%
Vietnam	UNX 1897	13	Cinnamic aldehyde 53%, 2-Propen-1-ol, 3-phenyl-, acetate 21%
Lao PDR	UNX 1793	36	Camphor 20% 1,8-Cineole (Eucalyptol) 20% 1- $\alpha$ -Terpineol 8%
Lao PDR	UNX 1898b	93	Myristicin 7%
Lao PDR	UNX 1898d	99	-
Lao PDR	UNX 1898e	82	Asarone 5% Myristicin 10%
Indonesia	UNX 1899a	11	Eugenol 37% $\alpha$ -Pinene 33% Methyleugenol 2%
Indonesia	UNX 1899b	0	Eugenol 45% $\alpha$ -Terpinolene 29% Methyleugenol 5%
Indonesia	UNX 1899c	100	-
Myanmar	UNX 1900	86	Asarone 8% Myristicin 5%

\* Content was estimated from the GC-MS area percentage of safrole and other compounds.

The concentration ranges for some of the key compounds were as follows:

Asarone 3-8%	Myristicin 5-10%
Camphor 2-42%	$\alpha$ -Pinene 33%
1,8-Cineole (Eucalyptol) 3-21%	2- $\beta$ -Pinene 21%
Cinnamic aldehyde 53%	<b>Safrole 0-100%</b>
Eugenol 37-45%	1- $\alpha$ -Terpineol 5-8%

## 1.2. Reference substances tested

Several other compounds present in essential oils are of special interest concerning their influence on the colour reactions. To study possible interferences with the colour reagents used for identification of safrole, the most relevant compounds identified by GC-MS were tested individually with all available colour reagents. Special focus was given to substances with molecular structures similar to safrole (for structures, see annex 3, p.6-7). The reference substances tested were those in Table 2 below.

Table 2: List of reference substances

Terpenes	Substituted allylbenzenes, 2-propenylbenzenes and aldehydes
Borneol	Anethol
D-Camphor (flowers)	p-Anisaldehyde
DL-Camphor (synthetic)	$\alpha$ -Asarone
(+)-Camphor-10-sulfonic acid	$\beta$ -Asarone
Cedar oil	Cinnamic aldehyde
1,8-Cineol (Eucalyptol)	Eugenol
Citronella oil	Isoeugenolmethylether
D-Limonene ((R)-(+)-Limonene)	Isosafrole
L-Limonene ((S)-(-)-Limonene)	Methyleugenol
Linalool	Myristicin
Linalool (synthetic)	Piperonylmethylketone (PMK)
$\alpha$ -Phellandrene	Safrole
(1)-(S)-(-)- $\beta$ -Pinene	
Terpineol	
Terpinol (menthadienes and cineoles)	

## 2. Colour tests

The following colour test reagents were used:

1. Marquis reagent
2. Gallic acid
3. Concentrated sulfuric acid
4. Froehde reagent
5. Mandelin reagent
6. Mecke reagent
7. Phloroglucinol

In addition, reagents from the UNODC drug and precursor test kits were tested with pure safrole.

## 2.1. Preparation of reagents

### Marquis Reagent [4]

Reagent A1: Add 8-10 drops (approx. 0.25 ml) of 37% formaldehyde solution to 10 ml glacial acetic acid.

Reagent A2: concentrated sulfuric acid

### Gallic Acid Reagent [4]

Dissolve 0.5 g of gallic acid in 100 ml concentrated sulfuric acid.

Gallic acid reagent was found to degrade slowly turning the reagent pale violet. To make the reagent more stable, gallic acid was dissolved in absolute ethanol and the sulfuric acid was directly added on the spot plate (2-component reagent).

### Mecke Reagent [4]

Dissolve 1.0 g of selenious acid in 100 ml of concentrated sulfuric acid.

### Froehde Reagent [5]

Dissolve 0.5 g molybdic acid or sodium molybdate in 100 ml of hot concentrated sulfuric acid.

### Mandelin Reagent [5]

Dissolve 1.0 g of ammonium vanadate in 100 ml of concentrated sulfuric acid.

### Phloroglucinol

Reagent 1: Dissolve 50 mg of phloroglucinol (1,3,5-trihydroxybenzene) in 5 ml of absolute ethanol.

Reagent 2: Concentrated hydrochloric acid.

Phloroglucinol can be used for the detection of methylenedioxy-groups. [6, "Phloroglucin"]  
The phloroglucinol reagent was initially prepared by dissolving 1% of substance in 6N hydrochloric acid, as described in literature [7, p. 625]. However, this solution was not stable and turned yellow within a week. Phloroglucinol dissolved in concentrated sulfuric acid also turned yellow and later orange. Phloroglucinol dissolved in glacial acetic acid or absolute ethanol was stable. There was no difference between the acetic acid solution and the alcoholic solution regarding the colour produced. The concentrated hydrochloric acid is stored in a separate bottle (2-component reagent).

### Reagents from the UNODC drug and precursor test kits

Reagents included in the UNODC drug and precursor test kits (namely tests A, C, D, E, G, O, T and U) were also used to test pure safrole. As expected, no colour reactions were observed with exception of test A – Marquis test. A list of all tests can be found in: [4].

Blanks were done using pure ethanol. No colour reactions were observed.

## 2.2. Colour test procedure

Using a disposable pipette, one drop of the diluted (at about 5 mg/ml in absolute ethanol) reference substance or essential oil was transferred into the depression of a white, ceramic spot plate. For pure, undiluted samples, an inoculating loop (see below) was used for this purpose.

Sample spots were then mixed with the appropriate amount of reagent(s). Each analysis was carried out in duplicate using adjacent depressions.

### 2.3. Observation of colour test results

Colours were observed immediately after adding the reagents and after 60 seconds. During this period colours usually stayed the same or got slightly darker. The colour reactions were described with words and documented by taking pictures.

The documentation of the spots of each colour test was carried out by taking pictures with a NIKON L1 digital camera. The digital camera was set in macro mode and the spots were put under artificial light (neon lamps). Depending on the type of camera and printer, the colours of the spots differ slightly.

## 3. Sample preparation

### 3.1. Sample preparation:

#### Optimizing the amount of safrole or safrole-rich oils used for colour testing

Small amounts (less than the “size of a match head” or “as little as possible”) of undiluted pure safrole were tested with Marquis and gallic acid reagent (annex 3, p. 1). The spot-tests produced very dark, almost black purple/violet colours that were difficult to distinguish.

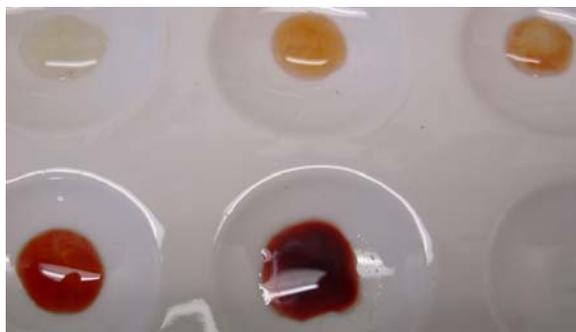


Figure 3: Gallic acid test with different safrole concentrations

Therefore, solutions of safrole in absolute ethanol at 0.1 mg/ml, 1.0 mg/ml, 5.0 mg/ml, 10 mg/ml and 25 mg/ml were prepared to identify the most suitable concentration for colour observation.

The investigation showed that a concentration range of 5-10 mg/ml safrole in absolute ethanol is reasonable for spot-tests with Marquis reagent and gallic acid test (annex 3, p. 1).

Based on these experiments, the reference standards were all prepared at a concentration of **5 mg/ml**. From these solutions one drop was used for spot plate analysis.

Results for phloroglucinol showed that this reagent is better used with undiluted samples (annex 3, p. 1-2).

### 3.2. Sample preparation:

#### Identifying the most suitable solvent for sample dilution

Other solvents than ethanol were tested. They included toluene, xylene (mixed isomeres), n-hexane, dichloromethane (DCM), ethyl acetate, 2-propanol, 1-butanol, 2-butanone (ethyl methyl ketone, MEK), and acetonitrile.

The first step of testing the suitability of the different solvents included a blank containing no safrole (1 drop of solvent mixed with the available reagents). Toluene and xylene were found to produce a red precipitate with Marquis reagent (actually used to identify toluene) and were therefore considered unsuitable for any further use.

In a second step, solutions of safrole (5 mg/ml) were prepared using the solvents n-hexane, dichloromethane (DCM), ethyl acetate, 2-propanol, 1-butanol, 2-butanone (MEK) and acetonitrile. These solutions were tested and the colour reactions were compared.

With these solvents only very slight differences in colour (regarding the intensity, not the hue) were observed. Thus absolute ethanol was used for dilutions because of its low toxicity.

Another method to dilute the samples involved adding a small amount of oil to a comparatively large amount of distilled water and shaking until a turbid emulsion was formed. Safrole-concentrations of 0.1-25 mg/ml H<sub>2</sub>O were tested, 5-25 mg/ml worked well. That means one drop of oil (~ 50 mg) is added to 2-10 ml of water. From this emulsion one drop was taken immediately after shaking and used for testing. The emulsion will separate after some time and has to be shaken again. This dilution can be useful when no ethanol is available.



Figure 4: Emulsion of safrole in water

It is also possible to dilute oil samples with water directly on the spot plate. Apply a small amount of substance (very little is needed), add one drop of water and stir well using a glass rod or inoculation loop. Then add the reagent(s). However, dilutions with alcohol or emulsions produced in small bottles or flasks will work better.

Tap water can be used when no distilled water is available. In this case a blank has to be done to make sure the water doesn't interfere with the test.

### 3.3. Colour test sample preparation

Samples of pure reference substances (safrole and other compounds found in safrole-containing essential oils, i.e., different terpenes, substituted allylbenzenes and 2-propenylbenzenes) were prepared at a concentration of 5 mg/ml in absolute ethanol.

Essential oil samples were diluted with absolute ethanol at 5 mg/ml. From each solution one drop was applied to the depression of the spot plate using a disposable pipette. Then the appropriate amount of reagent(s) was added.

The undiluted oils were also investigated because absolute ethanol and analytical scales are usually not available in field situations. However, to test undiluted oils only very little substance (far less than one drop) is needed. Dipping a spatula into the oil and then touching the spot plate does not allow the transfer of reproducible amounts of oil. Therefore, a small inoculating loop was made from a piece of steel wire (e.g., a paper clip). This little tool, which should be available everywhere, was sufficient for transferring roughly reproducible amounts of oil to the spot plate.



Figure 5: Inoculating loop

In case no organic solvents are available water can be used to dilute samples. Water and oil samples will not mix but strong shaking forms an emulsion which can be used to carry out colour reactions (see chapter 3.2).

## RESULTS & DISCUSSION

### 4. Results of spot plate analysis

#### 4.1. Reference substances

Preliminary tests showed quickly that only four colour tests deserved further study during the project, namely: Marquis test, gallic acid test, concentrated sulfuric acid and phloroglucinol test.

Table 3 summarizes the resulting colours of substances found in essential oils and their concentration in the samples. The corresponding colour photo series is given in annex 3, p. 3-8.

Table 3: Colour test results of reference substances

Reference substance	% in oil samples *	Marquis test	Gallic acid test	H <sub>2</sub> SO <sub>4</sub> conc.	Phloroglucinol
<b>Terpenes (5 mg/ml in abs. EtOH)</b>					
D-Camphor (flowers)	2-42	-	-	-	-
1,8-Cineol (Eucalyptol)	3-21	orange	yellow	dark yellow	-
D-Limonene ((R)-(+)-Limonene)	1-3	orange	dark yellow	yellow	-
L-Limonene ((S)-(-)-Limonene)	1-3	pale orange	dark yellow	bright orange	-
Linalool	4	orange	greenish yellow	bright yellow	-
(1S)-(-)-β-Pinene	21	orange	dark yellow	bright orange	-
Terpineol	5-8	pale orange	dark yellow	bright orange	-
<b>Substituted allylbenzenes, 2-propenylbenzenes and aldehydes (5 mg/ml in abs. EtOH)</b>					
Anethole	-	brown	pale orange	pale orange	-
p-Anisaldehyde	-	pale brown	pale brown	pale brown	intense redd. orange
α-Asarone	3-8	yellow	orange	intense yellow	-
β-Asarone	3-8	yellow	dark yellow	intense yellow	-
Cinnamic aldehyde	53	pale beige	pale greenish yellow	pale yellow	intense orange red
Eugenol	37-45	<b>dark purple</b>	redd. orange	redd. orange	<b>pink</b>
Isosafrole	-	dirty violet	redd. orange	red	-
Methyleugenol	2-5	<b>purple</b>	pinkish orange	orange	<b>pale pink</b>
Myristicin	5-10	brown	orange	orange	<b>pink</b>
<b>Safrole</b>	0-100	<b>blackish blue to dark violet</b>	<b>red to purple</b>	<b>dark purple</b>	<b>pink</b>

\* Content estimated from the GC-MS area percentage of the analyzed oils.

Legend: Hyphen (-) indicates "no colour"

#### 4.1.1. Findings

##### Terpenes:

- None of the tested terpenes showed colours similar to those of the safrole colour test results.
- Safrole produces intense dark blue, red or purple colours depending on the reagent used. The terpenes produce yellow and orange colours. However, these hues are not strong enough to disguise, for example, the blackish blue-to-violet colour from the reaction of safrole with the Marquis reagent.
- Within the terpenes special attention was given to camphor, 1,8-cineole and the pinenes, because of their relative high concentrations in some of the analyzed natural oil samples:
  1. D-Camphor produced no colour reaction with any of the above reagents.
  2. 1,8-Cineole (eucalyptol) showed yellow to orange colours.
  3.  $\beta$ -Pinene also produced yellow and orange hues.These compounds will not cover colours produced by safrole.
- With the tested terpenes the phloroglucinol reagent gave no colour reaction at all.

##### Methoxy-/methylenedioxy-substituted allylbenzenes and 2-propenylbenzenes and aldehydes:

- Eugenol and methyleugenol gave dark purple and purple colour reactions with Marquis reagent.
- Methyleugenol was present in two samples at low concentrations (2-5%). It was tested because of its structure being similar to that of safrole.
- The asarones (3-8% in oil samples) produced yellow and orange hues unlikely to interfere with safrole detection.
- Myristicin (5-10% in oil samples) has shown a brown colour with the Marquis reagent and an orange colour with the gallic acid test and concentrated sulfuric acid.
- Asarone and myristicin were found only in oil samples that were specified as very rich in safrole.
- Cinnamic aldehyde (53% in one sample) and p-anisaldehyde (not found in any sample) gave pale brown and pale yellow colours with the Marquis reagent, the gallic acid and the concentrated sulfuric acid. Examinations of these two aldehydes with the phloroglucinol reagent produced an intense reddish orange colour, in line with expectations for this reagent.
- Isosafrole produced no colour with phloroglucinol reagent.
- The phloroglucinol reagent alone cannot differentiate between safrole and eugenol. However, in combination with Marquis reagent and  $H_2SO_4$  conc. a distinction is possible (see annex 3, p.8).

##### Summary of results using other colour test reagents

A number of other reagents were also tested because their use for the detection of drugs of abuse or precursor materials has been described in the literature.

Froehde reagent: All substituted aromatic compounds (including the aldehydes) and most of the terpenes produced very dark blue colours. Camphor gave a pale blue colour while (+)-camphor-10-sulfonic acid did not react at all.

Mandelin reagent: With eugenol and methyleugenol dark brownish purple hues were found. Myristicin produced a brown colour and with safrole a very dark purple/violet resulted. When Mandelin reagent was added to the terpenes generally colours similar to those produced with  $H_2SO_4$  conc. (or slightly darker) were observed.

Mecke reagent: Eugenol and methyleugenol gave dark greenish blue colours. Myristicin produced a blackish blue and safrole resulted in a dark bluish green. With most terpenes brown hues were found.

Reagents from the UNODC drug and precursor test kits: Only pure safrole was tested and did not result in a colour change with any of the included reagents except reagent A (Marquis reagent).

## 4.2. Natural oil samples

From all samples which have been analyzed by GC-MS only the most interesting ones were chosen. These included all oils that contained high levels of other substances in addition to safrole and some oils that consisted of almost pure safrole.

### 4.2.1. Oil samples diluted with absolute ethanol

Table 4 summarizes the colours resulting from essential oil samples diluted with absolute ethanol (5 mg/ml), their safrole content and levels of other compounds. The corresponding colour photo series is given in annex 3, p. 9-11.

Table 4: Colours resulting from essential oils diluted with absolute ethanol

Sample Code	% Safrole *	other compounds *	Marquis test	Gallic acid test	H <sub>2</sub> SO <sub>4</sub> conc.	Phloroglucinol
UNX 1897	13	Cinn. aldehyde 53% 2-Propen-1-ol, 3-phenyl-, acetate 21%	pale orange/ brown	dirty orange	orange	intense reddish orange
UNX 1898e	82	Asarone 5% Myristicin 10%	brownish purple/violet	reddish orange	reddish purple	pink
UNX 1899a	11	Eugenol 37% $\alpha$ -Pinene 33%	dark purple	pale reddish orange	pale reddish orange	pink
UNX 1899b	0	Eugenol 45% $\alpha$ -Terpinolene 29%	dark purple	yellow	yellow	pink
UNX 1900	86	Asarone 8% Myristicin 5%	brownish purple/violet	reddish orange	reddish purple	pink
UNX 1902a	15	Camphor 42 % 1,8 Cineole 21% 1- $\alpha$ -Terpineol 5%	pale greenish brown	greenish yellow	pale orange	-
UNX 1902b	92	Asarone 3% Myristicin 2%	blackish blue/ dark violet	reddish orange	purple	pink
UNX 1902c	0	$\alpha$ -Cedrol 9% unknown 53%	brown	-	pale yellow	-
UNX 1902d	84	Benzaldehyde 6% $\alpha$ -Terpinene 4%	blackish blue/ dark violet	orange	purple	pale pink
UNX 1902e	42	Camphor 4% 1,8-Cineole 18% $\alpha$ -Pinene 10 % 2- $\beta$ -Pinene 21 %	greyish blue	reddish orange	reddish orange	pale pink
UNX 1902f	91	-	blackish blue/ dark violet	reddish orange	reddish purple	pink
UNX 1902g	96	Limonene 3%	blackish blue/ dark violet	red	dark reddish purple	pink
UNX 1902h	97	-	blackish blue/ dark violet	red	reddish purple	pink

\* Content estimated from the GC-MS area percentage of the analyzed oils.

Legend: Hyphen (-) indicates "no colour"

#### 4.2.2. Findings

##### Marquis reagent:

- Natural oils rich in safrole (> 80%) gave the same blackish blue/dark violet colour with Marquis reagent like pure safrole.
- One sample (UNX 1898e) containing 82% of safrole along with 10% myristicin and 5% asarone produced a brownish purple/violet colour with Marquis.
- Samples containing 0-15% safrole failed to produce the typical blackish blue/violet Marquis reaction for safrole.
- UNX 1899a (11% safrole, 37% eugenol) and UNX 1899b (0% safrole, 45% eugenol) gave a dark purple colour.

##### Gallic acid test and H<sub>2</sub>SO<sub>4</sub> conc.:

- These two reagents produced similar colours with the same oil sample. Typically red and purple hues were found with safrole-rich oils. The colour intensity increased with the safrole content. Samples containing no safrole and no eugenol gave no colour or just a pale yellow (e.g., UNX 1902c).

##### Phloroglucinol reagent:

- Because rather pale pink colours were produced with diluted samples, the phloroglucinol test is better used with pure oils.
- Cinnamic aldehyde (present in one sample at 53%) and p-anisaldehyde (not found but tested) gave intense orange/red colours which can cover pink hues resulting from safrole in the same oil.

#### 4.2.3. Undiluted (pure) oil samples

Table 5 summarizes the colours resulting from pure essential oil samples, their safrole content and levels of other compounds. The corresponding colour photo series is given in annex 3., p. 12-14).

Table 5: Colours resulting from undiluted (pure) essential oil samples

Sample Code	% Safrole *	% other compounds *	Marquis test	Gallic acid test	H <sub>2</sub> SO <sub>4</sub> conc.	Phloroglucinol
UNX 1793	36	Camphor 20% 1,8-Cineole 20 % 1- $\alpha$ -Terpineol 8%	dark brownish purple	orange red	dark reddish purple	pink
UNX 1897	13	Cinn. aldehyde 53% 2-Propen-1-ol, 3-phenyl-, acetate 21%	dark yellow	dark dirty yellow	light brown	intense reddish orange
UNX 1898e	82	Asarone 5% Myristicin 10%	v.d. blackish violet	v.d. red to purple	dark purple	pink
UNX 1899a	11	Eugenol 37% $\alpha$ -Pinene 33%	v.d. purple	purplish red	dark purple	pink
UNX 1899b	0	Eugenol 45% $\alpha$ -Terpinolene 29%	dark purple	orange	dark orange red	pink
UNX 1900	86	Asarone 8% Myristicin 5%	v.d. blackish violet	v.d. red to purple	purple	pink
UNX 1902a	15	Camphor 42 % 1,8-Cineole 21 % 1- $\alpha$ -Terpineol 5%	brown	pale reddish orange	reddish brown	very pale pink

Sample Code	% Safrole *	% other compounds *	Marquis test	Gallic acid test	H <sub>2</sub> SO <sub>4</sub> conc.	Phloroglucinol
UNX 1902b	92	Asarone 3% Myristicin 2%	v.d. blackish blue/violet	dark red to dark purple	v.d. purple/red	intense pink
UNX 1902c	0	$\alpha$ -Cedrol 9% unknown 53%	orange brown	yellow	brownish orange	reddish orange
UNX 1902d	84	Benzaldehyde 6% $\alpha$ -Terpinene 4%	v.d. blackish blue/violet	dark red to dark purple	v.d. purple/red	pale pink
UNX 1902e	42	Camphor 4% 1,8-Cineole 18% $\alpha$ -Pinene 10 % 2- $\beta$ -Pinene 21 %	d. blackish blue/violet	dark red to dark purple	v.d. purple/red	pale pink
UNX 1902f	91	-	v.d. blackish blue/violet	dark red to dark purple	v.d. purple/red	pale pink
UNX 1902g	96	Limonene 3%	v.d. blackish blue/violet	dark red to dark purple	v.d. purple/red	pink
UNX 1902h	97	-	v.d. blackish blue/violet	dark red to dark purple	v.d. purple/red	pink

\* Content estimated from the GC-MS area percentage of the analyzed oils.

Legend: v.d. = very dark

#### 4.2.4. Findings

##### Marquis reagent:

- Samples rich in safrole (> 80%) produced very dark blackish blue/violet colours similar to pure safrole.
- One sample (UNX 1898e) containing 82% of safrole along with 10% myristicin and 5% asarone produced a brownish purple/violet colour with Marquis reagent.
- UNX 1899a (11% safrole, 37% eugenol) gave a very dark purple hue similar to UNX 1899b (0% safrole, 45% eugenol), which was dark purple.
- UNX 1902c (0% safrole, 0% eugenol) resulted in an orange brown colour when treated with Marquis reagent.

##### Gallic acid test and H<sub>2</sub>SO<sub>4</sub> conc.:

- Safrole-rich oils (> 80%) produced the typical dark red/purple tones also obtained from pure safrole. These two reagents produced similar colours with the same oil sample.
- UNX 1899b (0% safrole, 45% eugenol) gave orange/dark orange red colours. UNX 1902c (0% safrole) resulted in yellow and brownish orange tones.

##### Phloroglucinol reagent:

With all samples a colour reaction occurred. It was always pink (intensity varied) with two exceptions:

- UNX 1897 (13% safrole, 53% cinnamic aldehyde) gave an intense reddish orange colour which covered the pink hue associated with safrole. Cinnamic aldehyde can be recognized by its strong cinnamon smell.
- UNX 1902c (0% safrole) produced a reddish orange colour.

#### 4.2.5. Safrole cut-off concentration

The above analyses determined a safrole concentration of about 20% as the cut-off below which colour tests did not consistently produce positive results.

### 5. Discussion and conclusions

Samples of safrole and safrole-rich essential oils were systematically studied (and results systematically documented) using different colour tests. Existing tests were improved to ensure availability of the most effective tests for the field. Simple modifications were proposed regarding the specificity and stability of reagents.

Specifically, the following improvements are suggested:

- Use of a simple tool (“**inoculating loop**”) made from a piece of steel wire (e.g., a paper clip) to improve the reproducibility of the amounts of oil transferred to the spot plate
- **Dilution of oil samples** with absolute ethanol to improve colour recognition with most colour tests. A safrole concentration of 5-10 mg/ml is recommended. Such concentration is obtained by dissolving one drop of oil in about 10 ml of absolute ethanol. Water is another possible solvent for diluting oil samples. However, since the oils and water are not miscible, tests have to be carried out on the emulsions, before phase separation, to achieve reproducible results.
- Use of **additional reagents** to improve the differentiation of safrole-rich essential oils and samples containing little or no safrole:
  - Gallic acid reagent: ethanolic gallic acid solution and concentrated sulfuric acid (in two separate reagent bottles, i.e., as a two-component reagent, to increase stability of the reagent)
  - Concentrated sulfuric acid
  - Phloroglucinol reagent: ethanolic phloroglucinol solution and concentrated hydrochloric acid (in two separate reagent bottles, i.e., as a two-component reagent, to increase stability of the reagent)

In terms of test results from the reference substances, the following can be concluded:

- Terpenes do not interfere with any safrole colour test.
- Camphor, which is often accompanying safrole in natural oils, does not interfere with any of the four colour tests.
- Using the phloroglucinol reagent, a distinction can be made between (I) aldehydes (intense orange-red colour), (II) anethole, asarones, isosafrole (no colour) and (III) the group of eugenol, methyleugenol, myristicin and safrole (pink).
- The phloroglucinol reagent alone cannot differentiate between safrole, eugenol, methyleugenol and myristicin.
- Eugenol, methyleugenol and myristicin can be distinguished from safrole using the Marquis reagent and concentrated sulfuric acid (see Annex 3, p.8).
- A safrole content of about 20% can be considered the cut-off for positive safrole determination with the four colour tests.

In terms of colour tests, the following reagents can be used for the identification of safrole in safrole-containing essential oils:

#### Marquis reagent:

The Marquis reagent has produced intense dark blue, dark violet and dark purple colours with the *substituted allylbenzenes* safrole, eugenol and methyleugenol. The reagent can be used for these substances:

- Isosafrole (3,4-methylenedioxy-2-propenylbenzene) resulted in purple/violet hues.
- Isoeugenolmethylether (a substituted 2-propenylbenzene) also gave a purple hue. However, this substance has not been identified in any of the oil samples analyzed.
- The spots of Marquis reagent with the selected *terpenes* have shown either no colour (e.g., camphor) or only yellow, orange and brown colours (e.g., 1,8-cineole) were obtained.

The Marquis reagent was found to give very dark blackish hues with pure samples. If very dark colours are produced, it is recommended to repeat the test with a smaller amount of sample, or to further dilute the sample.

#### Gallic acid test:

This reagent reacts specifically with *methylenedioxy-substituted aromatic compounds*. [8, p.18]

- It was found to give a red colour reaction when added to safrole, a reddish-orange with isosafrole, a pinkish-orange with methyleugenol, an orange colour with myristicin and a yellow colour with the asarones.
- With the tested *terpenes* either no colour (e.g. camphor) or only orange and yellow hues (e.g. 1,8-cineole) were obtained.

#### Concentrated sulfuric acid:

H<sub>2</sub>SO<sub>4</sub> conc. was used because it is part of Marquis reagent (reagent A2) and thus included in the UN precursor and drug test kit.

- Concentrated sulfuric acid has produced a purple hue with safrole, a red hue with isosafrole, a reddish orange colour with eugenol and an orange hue with methyleugenol and myristicin.
- *Terpenes* generally gave yellow colours with concentrated sulfuric acid.
- When H<sub>2</sub>SO<sub>4</sub> conc. and gallic acid test reagent were added to safrole side by side on the same spot plate, not much colour difference was seen.
- In comparison to gallic acid reagent, the H<sub>2</sub>SO<sub>4</sub> conc. alone gave slightly more purple hues.

#### Phloroglucinol reagent:

This reagent produced pink colours with eugenol, methyleugenol, myristicin and safrole. With isosafrole and anethole it produced a pale violet and pale pink colour, respectively, and can therefore be used to differentiate between the side chain isomers safrole and isosafrole.

- With the tested *aldehydes* intense reddish orange and red hues were obtained.
- All *terpenes* which were tested with this reagent failed to produce a visible colour change.
- With diluted samples, the phloroglucinol reagent results in rather pale shades of pink. It is therefore recommended to use it preferably with pure oils and standards.

#### Smell

The characteristic smell of safrole can contribute to the positive identification of safrole-rich essential oils.

### Reaction mechanisms:

Taking into account the colour test results from the reference substances and their chemical structures, some speculations about the reaction mechanisms were carried out. What appears to be clear is that:

- the phloroglucinol reagent results in a positive test result (i.e., coloured spot) with allyl (= 2-propenyl) side chains (cf: safrole = pink; isosafrole with a 1-propenyl side chain = no colour). [10, p. 169]
- the formation of formaldehyde from the action of concentrated sulfuric acid on the methylenedioxy-group plays a central role in several colour tests [11] and provides useful results even when used on its own (cf: gallic acid test vs. concentrated sulfuric acid).

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## Pictures

All pictures in the annex were taken by Andreas Leitner.

## Structural formulas

All structural formulas (in the annex) were created using Isis Draw 2.3.

## ANNEXES

### ANNEX 1: SUGGESTED OPERATING PROCEDURE

#### Colour test procedure for identification of safrole-rich essential oils

Recommendation: Familiarize yourself with the procedures and colour test results using standard reference substances and/or reference samples before testing real samples.

Description for preliminary identification:

- 1) Thoroughly clean all equipment.
- 2) Blank (negative control): Mix the amounts of reagents needed for each test (e.g., one drop A1 + three drops A2 for Marquis test) within the depressions of spot plate. Do not add any sample. No colour reaction must occur.
- 3) Dip inoculation loop into the unknown sample and smear the substance onto the surface of spot plate within one depression. Dip once again and smear it on the next spot for duplicate testing. Test the sample with each colour reagent (it is recommended to test samples simultaneously, on the same spot plate, with all four reagents. Observe and document the immediate colour reaction and any changes within 60 seconds. Compare the colours to previous tests and published colour tests (see annex 3).
- 4) Thoroughly clean all equipment.

#### Blank, standard reference substance, reference sample

**Blank (negative control)**: Mix appropriate amounts of reagents on spot plate without adding any substance (oil). There must not be any colour reaction.

**Standard reference substance**: specified safrole (e.g., assay > 97%)

Usage: Colour tests are done with specified safrole to see the actual colour reaction under comparable conditions.

**Reference sample**: specified safrole-rich oil (composition of the safrole-rich oil has been determined via GC-MS).

Usage: Colour tests are done with a specified safrole-rich oil to see the actual reaction under comparable conditions.

#### Equipment

**Spot plate**: e.g., white ceramic spot plate (plastic will soon discolour because of corrosive reagents). Spot plates with 8 or 12 depressions are preferred to conduct colour tests with all four reagents next to each other for colour comparison. The standard reference substance

(specified safrole) or the sample reference (specified safrole-rich oil) should be tested with the above reagents next to the unknown sample to compare the results.

**Transfer tools:**

a) for pure oils: an inoculation loop is used

b) for diluted oil samples: a (disposable) pipette is used

Inoculation loops and pipettes which are used more than one time must be cleaned thoroughly after each use to prevent cross-contamination (e.g., acetone for cleaning inoculation loops) and a blank should be done using cleaned equipment prior to every use on real samples.

**In addition:** Tissues, glass rods, small vials and beakers.

<b>Colour test reagents</b>
-----------------------------

Colour test reagent 1: **MARQUIS REAGENT**

UN precursor test kit reagent A1: ~ 0,25 ml 37% formaldehyde in 10 ml glacial acetic acid

UN precursor test kit reagent A2: concentrated sulfuric acid

Drops used for test:

1 drop of A1, then 3 drops of A2

Colour test reagent 2: **SULFURIC ACID**

UN precursor test kit reagent A2: concentrated sulfuric acid

Drops used for test:

1 drop of A2

Colour test reagent 3: **GALLIC ACID**

~ 25 mg gallic acid (3,4,5-trihydroxybenzoic acid) dissolved in 5 ml absolut ethanol and concentrated sulfuric acid in separate bottle

Drops used for test:

1 drop of gallic acid solution, then 1 drop of concentrated sulfuric acid (reagent A2)

Colour test reagent 4: **PHLOROGLUCINOL**

~ 50 mg phloroglucinol (1,3,5-trihydroxybenzene) dissolved in 5 ml absolute ethanol and concentrated hydrochloric acid in separate bottles

Drops used for test:

1 drop of phloroglucinol solution, then 1 drop of concentrated hydrochloric acid

<b>General observations / remarks on colours</b>
--------------------------------------------------

Colours usually turn darker with increased reaction time. Therefore, only results obtained within 60 seconds should be considered.

The intensity of the colour depends on the amount of substance used. To obtain comparable results, the same amount of substance has to be used.

Whenever very pale or very dark colours are produced the test should be repeated with more or less substance. This is especially important when testing pure substances (with solutions, every drop will hold sufficiently equal amounts of oil).

To test pure substances and pure essential oils, only very little substance (far less than one drop) is needed. Dipping a spatula into the oil and then touching the spot plate will not transfer reproducible amounts of substance. A small inoculation loop made from a piece of steel wire may be better suited for this purpose.

Spot plates must be cleaned thoroughly before each use. Contaminations can impede colour reactions or cause false positive results. To exclude contamination as the cause of a false positive result, blanks (aka negative controls) should be analyzed simultaneously to the sample.

Similarly, positive controls (using a sample of pure safrole or an oil which is definitely known for its high safrole content) could be analyzed on the same spot plate next to the unknown sample for colour comparison.

Different colours produced in repeated tests for one substance using the same reagent may have the following reasons:

- Different amounts of reagents were added to the sample.
- The amount of tested substance varied.
- The colours were observed at different times.
- Contamination of reagents or spot plates.

To obtain reproducible results all of the above factors must be considered. [9]

Individual colour perception and characterization by the operator has also to be considered.

The smell of safrole can contribute to the positive identification of safrole-rich essential oils.

## ANNEX 2: GAS CHROMATOGRAPHY – MASS SPECTROMETRY

### GC-MS operating conditions

Instrument: HP 5980 series II gas chromatograph with HP 5972 series mass selective detector

Capillary Column: DB-5, 30 m x 0.25  $\mu$ m x 0.25 mm

Carrier gas: Helium, flow 1.0 ml/min, septum purge 3 ml/min, split 1:50

Injection: 0.05  $\mu$ l, injector temperature 270°C, detector temperature 280°C

Temp. programm: initial temperature 80°C, oven rate 10°C/min to 270°C

Signal parameters: peak width 0.013, sampling rate 20 Hz

### GC-MS sample preparation

Essential oil samples were used without any further preparation. A volume of 0.05  $\mu$ L was injected with a Hamilton Microliter<sup>TM</sup> Syringe.

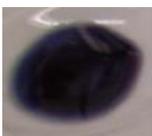
### ANNEX 3: COLOUR TEST PICTURES

Pure safrole (97%) + Marquis or gallic acid	1
Safrole (97%) – diluted (in abs. Ethanol) + reagents	1
Safrole dilution series + reagents	2
Terpene standards – diluted (in abs. ethanol) + reagents	3-5
Standards – diluted (in abs. ethanol) + reagents	6-7
Standards – pure + Phloroglucinol/HCl reagent	8
Eugenol and safrole – pure + Marquis or H <sub>2</sub> SO <sub>4</sub> c.: comparison	8
Natural oils - diluted (in abs. ethanol) + reagents	9-11
Natural oils - pure + reagents	12-14

### Safrole (97%) pure

$V_{\text{safrole}}$ [ $\mu\text{L}$ ]	Marquis	Gallic acid
1	 dark blue to dark purple/black	 dark purple
2	 dark blue to dark purple/black	 dark purple
3	 dark blue to dark purple/black	 dark purple
4	 dark blue to dark purple/black	 dark purple
5	 dark blue to dark purple/black	 dark purple
10	 dark blue to dark purple/black	 dark purple

### Safrole (97%) in abs. EtOH

safrole [mg/ml]	Marquis	Gallic acid	$\text{H}_2\text{SO}_4$ c.	Phloroglucino/HCl
0.1	 no colour	 slightly yellow (like blank)	 no colour	 no colour
1	 bluish grey	 orange	 pale orange	 very pale pink
5	 blackish blue/violet	 bright red/orange	 pale purple	 pale pink
10	 dark violet	 red	 purplish red/purple	 pink
25	 very dark violet/black	 dark red (like red vine)	 dark purplish red/purple	 pink

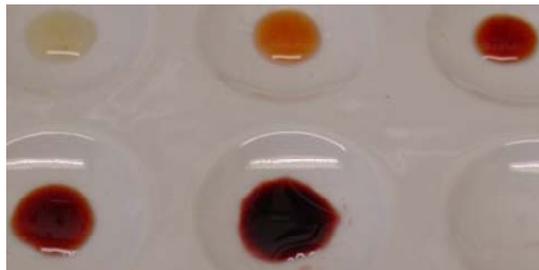
**Safrole (97%) in abs. EtOH**



**MARQUIS - REAGENT**

0.1	1.0	5.0
10.0	25.0	mg/ml

**Safrole (97%) in abs. EtOH**



**GALLIC ACID TEST**

0.1	1.0	5.0
10.0	25.0	mg/ml

**Safrole (97%) in abs. EtOH**



**PHLOROGLUCINOL/HCL**

0.1	1.0	5.0
10.0	25.0	mg/ml

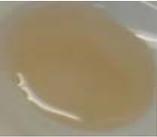
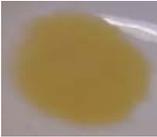
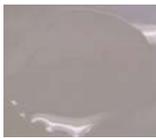
## Terpene standards

5mg/ml in abs. EtOH

substance	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloro/HCl
Borneol (BDH)	 pale yellow	 pale greenish yellow	 very pale green	 no colour
D-Camphor (flowers)	 no colour	 no colour	 no colour	 no colour
DL-Camphor (synth.)	 pale pink	 pale brown	 pale brown	 no colour
(+)-Camphor-10-sulfonic acid monohydrate	 no colour	 no colour	 no colour	 no colour
Cedar oil (DRAGOCO)	 brown	 pale orange	 pale orange	 no colour
1,8-Cineol	 orange	 yellow	 dark yellow	 no colour

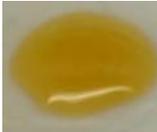
## Terpene standards

5mg/ml in abs. EtOH

substance	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloro/HCl
Citronell oil Java (DRAGOCO)	 pale orange/brown	 dark yellow/bright orange	 bright orange	 no colour
D-Limonene ((R)-(+)-Limonene, (ALDRICH))	 orange	 dark yellow/bright orange	 yellow	 no colour
(R)-(+)-Limonene	 dark orange	 dark yellow	 yellow	 no colour
L-Limonene ((S)-(-)-Limonene, (ALDRICH))	 pale orange/brown	 dark yellow/bright orange	 bright orange	 no colour
Linalool	 orange	 greenish yellow	 bright yellow	 no colour
Linalool, synth. (DRAGOCO)	 brown	 brownish orange	 yellow	 very pale yellow

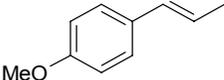
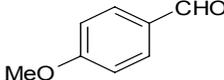
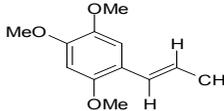
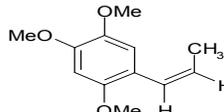
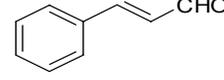
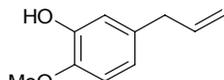
## Terpene standards

5mg/ml in abs. EtOH

substance	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloro/HCl
(1S)-(-)-β-Pinene (ALDRICH)	 orange	 dark yellow	 bright orange	 no colour
α-Phellandrene	 brown	 yellow	 yellow	 no colour
Terpineol, anhydr. (FLUKA)	 pale orange/ brown	 dark yellow/ bright orange	 bright orange	 no colour
Terpineol, 82% (DRAGOCO)	 dark orange/ brown	 dark yellow	 dark yellow	 no colour
Terpinol (menthadienes and cineoles, BDH)	 pale orange/ brown	 dark yellow/ bright orange	 orange	 no colour

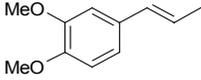
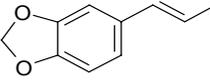
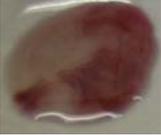
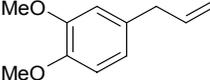
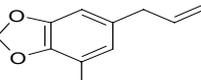
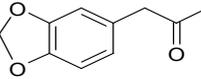
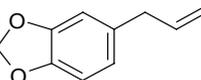
# Standards

5mg/ml in abs. EtOH

substance	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
Anethol 	 brown	 pale orange	 pale orange	 no colour
p-Anisaldehyde 	 greyish brown	 very pale grey/brown (like blank - reagent old)	 very pale brown	 intense reddish orange
α-Asarone 	 intense yellow to brown	 orange	 intense yellow/bright orange	 no colour
β-Asarone 	 bright yellow to orange	 dark yellow	 intense yellow	 no colour
Cinnamic aldehyde 	 pale beige	 greenish yellow	 yellow	 intense orange red
Eugenol 	 dark purple	 reddish orange	 reddish orange	 pink

# Standards

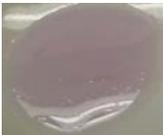
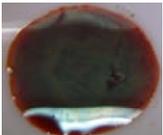
5mg/ml in abs. EtOH

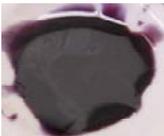
substance	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
Isoeugenol-methylether 	 pinkish red to dark purple	 pale orange	 orange	 no colour
Isosafrole 	 dirty violett	 reddish orange	 red	 no colour
Methyleugenol 	 purple	 pinkish orange	 orange (peach)	 pale pink
Myristicin 	 brown	 orange	 orange	 pink
Piperonylmethylketone (PMK) 	 green to bluish green	 orange	 reddish purple	 no colour
Safrole 	 blackish blue /dark violett	 red	 purple	 pink

## Pure standards

inoculating loop/spatula

Phloroglucinol in abs. EtOH (1 drop) + HCl c. (1drop)

<b>Anethole</b>		pale pink	<b>Isosafrole</b>		pale violet
<b>p-Anis-aldehyde</b>		intense reddish orange	<b>Methyleugenol</b>		pink
<b>α-Asarone</b>		yellow	<b>Cinnamic aldehyde</b>		dark red
<b>β-Asarone</b>		green	<b>Piperonyl-methyl-ketone (PMK)</b>		orange
<b>Eugenol</b>		intense pink	<b>Safrole</b>		intense pink
<b>Isoeugenol-methylether</b>		very pale green			

<b>Marquis</b>	<b>Eugenol</b>	<b>H<sub>2</sub>SO<sub>4</sub> c.</b>
	very dark purple	
<b>Marquis</b>	<b>Safrole</b>	<b>H<sub>2</sub>SO<sub>4</sub> c.</b>
	very dark blackish violet	

Since safrole and eugenol produce (see left) a similar colour with phloroglucinol/HCl reagent, Marquis reagent and concentrated sulfuric acid can be used for further differentiation.

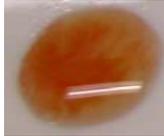
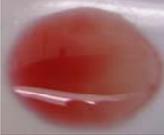
## "Safrole - containing Oils"

5mg/ml in abs. EtOH

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>China 1</b> UNX 1902a	15	1,8-Cineole 21% Camphor 42%	 pale dirty green/ brown	 greenish yellow	 pale orange	 no colour
<b>China 2</b> UNX 1902b	92	Asarone 3% Myristicin 2%	 blackish blue/ dark violet	 reddish orange	 purple	 pink
<b>China 3</b> UNX 1902c	0	α-Cedrol 9% unknown 53%	 brown	 no colour	 pale yellow	 no colour
<b>China 4</b> UNX 1902d	84	Benzaldehyde 6% α-Terpinene 4%	 blackish blue/ dark violet	 orange	 purple	 pale pink
<b>Safrole</b> (Aldrich)	97	-	 blackish blue	 red	 dark red /purple	 very pale orange

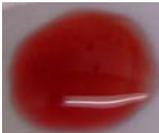
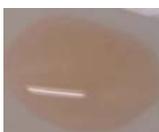
## "Safrole - containing Oils"

5mg/ml in abs. EtOH

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>China 6</b> UNX 1902e	42	α-Pinene 10% 2-β-Pinene 21% 1,8-Cineole 18%	 greyish blue	 reddish orange	 reddish orange	 pale pink
<b>China 7</b> UNX 1902f	91	unknown	 blackish blue/ dark violett	 reddish orange	 reddish purple	 pale pink
<b>China 8</b> UNX 1902g	96	DL-Limonene 3%	 blackish blue/ dark violett	 red	 dark reddish purple	 pale pink
<b>China 10</b> UNX 1902h	97	DL-Limonene 1%	 blackish blue/ dark violett	 red	 reddish purple	 pink
<b>Safrole</b> (Aldrich)	97	-	 blackish blue	 red	 dark red/ purple	 very pale orange

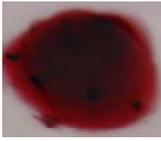
## "Safrole - containing Oils"

5mg/ml in abs. EtOH

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>UNX 1897</b> "Cicas Vietnam"	12	Cinnamic aldehyde 60% 2-Propen-1-ol, 3-phenyl,- acetate 21 %	 pale orange /brown	 dirty orange	 orange	 intense reddish orange
<b>UNX 1898e</b> "Phongsaly"	82	Asarone 5% Myristicine 10%	 brownish purple /violett	 red	 pinkish red	 very pale orange
<b>UNX 1899a</b> "Merpati Putih"	11	Eugenol 37% Pinene 33% Methyleugenol 3%	 dark purple	 pale reddish orange	 pale reddish orange	 pale pink
<b>UNX 1899b</b> "Manyak Lawang"	0	Eugenol 45% α-Terpinolene 29% Methyleugenol 5%	 dark purple	 yellow	 yellow	 pale yellow
<b>UNX 1900</b> "Cedar oil"	86	Asarone 8% Myristicine 6%	 brownish purple /violett	 reddish orange	 reddish purple	 very pale orange
<b>Safrole</b> (Aldrich)	97	-	 blackish blue	 red	 dark red /purple	 very pale orange

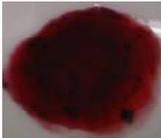
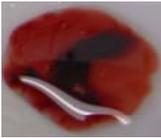
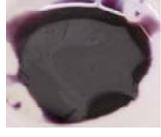
# Pure oils

inoculating loop/spatula

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>China 1</b> UNX 1902a	15	1,8-Cineole 21% Camphor 42%	 brown	 pale reddish orange	 reddish brown	 very pale pink
<b>China 2</b> UNX 1902b	92	Asarone 3% Myristicin 2%	 v. d. blackish blue/violet	 dark red to dark purple	 v. d. purple/red	 intense pink
<b>China 3</b> UNX 1902c	0	α-Cedrol 9% unknown 53%	 orange brown	 yellow	 brownish orange	 reddish orange
<b>China 4</b> UNX 1902d	84	Benzaldehyde 6% α-Terpinene 4%	 v. d. blackish blue/violet	 dark red to dark purple	 v. d. purple/red	 pink
<b>Safrole</b> (Aldrich)	97	-	 dark blue to dark purple/black	 dark purple	 very dark purple	 intense pink

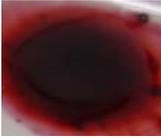
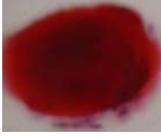
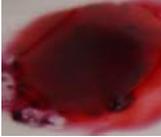
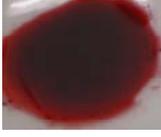
# Pure oils

inoculating loop/spatula

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>China 6</b> UNX 1902e	42	α-Pinene 10% 2-β-Pinene 21% 1,8-Cineole 18%	 dark blackish blue/ violet	 dark red to dark purple	 v. d. purple/ red	 pale pink
<b>China 7</b> UNX 1902f	91	unknown	 v. d. blackish blue/ violet	 dark red to dark purple	 v. d. purple/ red	 pale pink
<b>China 8</b> UNX 1902g	96	DL-Limonene 3%	 v. d. blackish blue/ violet	 dark red to dark purple	 v. d. purple/ red	 pink
<b>China 10</b> UNX 1902h	97	DL-Limonene 1%	 v. d. blackish blue/ violet	 dark red to dark purple	 v. d. purple/ red	 pink
<b>Safrole</b> (Aldrich)	97	-	 dark blue to dark purple/black	 dark purple	 very dark purple	 intense pink

# Pure oils

inoculating loop/spatula

	Safrole %	other compounds (GC-MS area %)	Marquis	Gallic acid	H <sub>2</sub> SO <sub>4</sub> c.	Phloroglucinol/HCl
<b>UNX 1793</b>	36	Camphor 20% 1,8-Cineole 20% 1- $\alpha$ -Terpineol 8%	 dark brownish purple	 orange red	 dark reddish purple	 pink
<b>UNX 1897</b> "Cicas Vietnam"	12	Cinnamic ald. 60% 2-Propen1-ol, 3-phenyl, acetate 21 %	 dark yellow	 dark dirty yellow	 light brown	 intense reddish orange
<b>UNX 1898e</b> "Phongsaly"	82	Asarone 5% Myristicin 10%	 very dark blackish violett	 very dark red to purple	 dark purple	 pink
<b>UNX 1899a</b> "Merpati Putih"	11	Eugenol 37% Pinene 33% Methyleugenol 2%	 very dark purple	 purplish red	 dark purple	 pink
<b>UNX 1899b</b> "Manyak Lawang"	0	Eugenol 45% $\alpha$ -Terpinolene 29% Methyleugenol 5%	 dark purple	 orange	 dark orange red	 pink
<b>UNX 1900</b> "Cedar oil"	86	Asarone 8% Myristicin 6%	 very dark blackish violett	 very dark red to purple	 purple	 pink