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Development of a simple field test for vehicle exhaust to detect illicit use of dyed diesel fuel

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ABSTRACT

Tax-free diesel fuel is intended for off-road uses such as agricultural operations, but illicit use of this fuel does occur and is a convenient way of evading payment of excise taxes. Current enforcement to prevent this practice involves visual inspection for the red azo dye added to the fuel to indicate its tax-free status. This approach, while very effective, has shortcomings such as the invasive nature of the tests and/or various deceptive tactics applied by tax evaders. A test designed to detect illicit dyed-fuel use by analyzing the vehicle exhaust would circumvent these shortcomings. This paper describes the development of a simple color spot test designed to detect the use of tax-free (i.e., dyed) fuel by analyzing the engine exhaust. Development efforts first investigated the combustion products of C.I. Solvent Red 164 (the azo dye formulation used in the United States to tag tax-free fuel). A variety of aryl amines were identified as characteristic molecular remnants that appear to survive combustion. A number of micro-analytical color tests specific for aryl amines were then investigated. One test that detected aryl amines by reacting with 4-(dimethylamino)benzaldehyde seemed to be particularly applicable and was used in a proof-of-principle experiment. The 4-(dimethylamino)benzaldehyde color spot test was able to clearly distinguish between engines that were burning regular fuel and those that were burning dyed diesel fuel. Further development will refine this color spot test to provide an easy-to-use field test.

1. Introduction

Tax-free fuel is legitimately used for agricultural and other off-road uses; however, its use on public roads can lead to loss of tax revenue. Although it is difficult to accurately estimate the revenue lost to fuel tax evasion, a somewhat dated source places the United States (U.S.) loss at billions of dollars annually [1]. Tax-free fuel in the U.S. is identified by the addition of 11.1 mg/L of C.I. Solvent Red 164 dye [2,3]. The chemical structure of a related dye (C.I. Solvent Red 26) is shown in Fig. 1. C.I. Solvent Red 164 is actually a mixture of dyes with an identical core structure as C.I. Solvent Red 26 except it has additional alkyl groups attached to the aromatic rings that result in enhanced solubility in diesel fuel [2]. The addition of C.I. Solvent Red 164 to fuel imparts a readily identifiable red coloration. Currently fuel tax enforcement personnel visually screen fuel for the characteristic red coloration of C.I. Solvent Red 164 at various inspection points. This screening approach has some shortcomings because of the invasive nature of the inspections and/or various deceptive tactics, such as the use of false fuel tanks and color masking, employed by tax evaders.

The overall goal of this research was to develop a simple, cost-effective test that could be performed on vehicle exhaust to determine if an engine is burning dyed, tax-free fuel. Because the test is intended for field use by personnel that are unlikely to have extensive backgrounds in chemical analysis, the test must be simple and reliable, and must provide unambiguous results. If successful, the approach would have numerous advantages over visual inspection of the fuel. Analyzing the exhaust rather than the fuel renders several deception techniques ineffective.

The objective of this study was to develop a simple field screening test that could be performed on diesel engine exhaust to determine if the engine is burning dyed fuel. Toward this end, thermal decomposition products from C.I. Solvent Red 164 produced under simulated combustion conditions (high temperatures in the presence of oxygen) were examined for characteristic dye remnants. If characteristic reaction products are formed, the study goal then becomes development of a micro-analytical color test that specifically targets the sensitive detection of relevant dye decomposition products in a format compatible with field testing. The final objective was to conduct a proof-of principle field trial based

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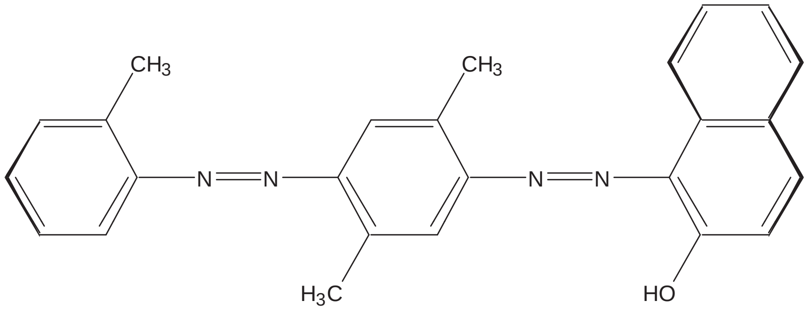


Fig. 1. Structure of C.I. Solvent Red 26. This dye has the same structural core as the dye used in the United States (C.I. Solvent Red 164) except the fuel dye contains additional alkyl groups to enhance its solubility in diesel fuel.

on the developed test to determine if the test could distinguish between exhaust from engines burning dyed fuel and those burning non-dyed diesel fuel.

2. Materials and methods

2.1. Chemicals, reagents, and materials

Methylene chloride, diethyl ether, isopropanol, 1,4-dioxane, and acetone solvents were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade benzene was obtained from Alfa Aesar (Ward Hill, MA, USA). Diethylenetriamine and 3,4-dimethylaniline were used as amine test probes and were obtained from Sigma–Aldrich. C.I. Solvent Red 164 (United Color Manufacturing, Newtown, PA, USA) was a subsample from a dye formulation batch sold to fuel terminals. The dye was stored in the dark as a 34% (w/v) xylene solution. The xylene solvent was removed immediately before use. Hydrochloric, phosphoric, and succinic acids as well as sodium hydroxide, also were obtained from Sigma–Aldrich. Sulfuric acid was purchased from Mallinckrodt (Paris, KY, USA) and acetic acid was obtained from Baker (Ultrex grade, Phillipsburg, NJ, USA). Deionized water was 18.2 MΩ-cm obtained from an in-house Milli-Q (Millipore Corporation, Bedford, MA, USA) system.

Color test reagents were obtained from a variety of vendors. The 4-(dimethylamino)benzaldehyde, *N,N*-dimethyl-4-nitrosoaniline, 1-(4-pyridyl)pyridinium chloride hydrochloride, hydroxylamine hydrochloride, and sodium nitroferricyanide(III) dihydrate (also known as sodium nitroprusside) were purchased from Sigma–Aldrich. The 2-furaldehyde and chloranil were manufactured by Fluka and purchased through Sigma–Aldrich. Fluorescamine was obtained from Pierce Chemical (Rockford, IL, USA).

Ehrlich's reagent was prepared by adding 1.5-g 4-(dimethylamino)benzaldehyde to 200 mL of a glacial acetic acid:methanol:water (5:3:2, v/v) mixture [4]. For a specific microplate reader application, the Ehrlich's reagent solvent mixture, without the added 4-(dimethylamino)benzaldehyde, was used.

Filter paper used for the color tests was 9-cm diameter #42 ashless filters (Whatman, Piscataway, NJ, USA). Spot tests were often visualized in porcelain spot plates (92 mm × 32 mm) that contained three depressions (VWR, West Chester, PA, USA). Dimethyldichlorosilane (DMCS)-treated glass wool was obtained from Alltech (Deerfield, IL, USA). Quartz tubes (6-mm o.d. × 4 mm i.d.) were purchased from McKisick Scientific Glass Blowing (Sumner, WA, USA). The tubes were washed with a dilute solution of micro-90 detergent (International Products, Burlington, NJ, USA) and thoroughly rinsed by successively aspirating solvents of decreasing polarity. Solvents used were water, acetone, isopropanol, and methylene chloride. Tubes were then dried before cutting into approximate 8-in. lengths.

2.2. Quartz tube oxidation

For this study, 10 mg of C.I. Solvent Red 164 dissolved in xylenes was deposited on a plug of silanized glass wool that was inserted approximately 3 in. into the bore of a quartz tube. A gentle flow of nitrogen gas was used to evaporate the xylenes, leaving the dye residue on the glass wool plug. The dye was then pyrolyzed by rapidly heating the quartz tube to 550 °C (by sliding a preheated tube heater over the pyrolysis tube) in the presence of an 11.5-mL/min air flow for 10-min. Thermal decomposition products were swept from the heated quartz tube and captured in an impinger that contained 20-mL methylene chloride.

2.3. Gas chromatography (GC)/mass spectrometry (MS) analysis

An Agilent 7980A gas chromatograph interfaced with a 5975C inert source mass-selective detector (MSD) was used for the analysis of the impinger solution. Separations were performed with a 1.2 mL/min constant flow of helium on a 30 m × 250 μm i.d. Rtx-5HT capillary column (Restek, Bellefonte, PA, USA). The Rtx-5HT column contained a 0.25-μm film of crosslinked 5% diphenyl, 95% dimethyl polysiloxane stationary phase. Samples (1.0 μL) were introduced into a 280 °C injector by splitless injection with a 0.8-min purge. After an initial 2 min hold at 50 °C, the column temperature was programmed to increase at a rate of 25 °C/min to 150 °C and then at a rate of 10 °C/min to a final temperature of 280 °C. The transfer line between the GC and MSD was maintained at 250 °C. Full-scan, 70-eV electron ionization mass spectra were obtained by scanning the MSD from *m/z* 50 to 550 at 50 Hz. Once collected, tentative identifications were provided by spectral matches with a National Institute of Standards and Technology 2005 mass-spectral database.

2.4. Color tests

Six visual color tests described by Feigl [5] and others [6] and a test based on fluorescence [7,8] were evaluated for their ability to detect aryl amines. Brief descriptions of these tests are provided below for quick reference. The tests were evaluated with 3,4-dimethylaniline dissolved in diethyl ether as a test standard, and were initiated using small quantities (typically 50–4 μg) of 3,4-dimethylaniline placed either in the color plate depression or on a reagent-containing filter.

(1) *Glutaconic aldehyde test*. The glutaconic aldehyde test involved adding one drop of 1-(4-pyridyl)pyridinium chloride hydrochloride reagent (1% aqueous solution, w/v) to the color plate depression followed by 1 drop of 1-M sodium hydroxide

to induce the *in situ* release of the reactive alkali glutaconic aldehyde intermediate. One drop of 12 M hydrochloric acid then was added, and the polymethine dye product formed with aryl amines was observed.

(2) *Furfural test*. The furfural test was preformed first on a color plate by adding 1 drop of furfural reagent. The reagent was prepared by adding 10 drops of furfural to 10-mL glacial acetic acid. Later, tests were conducted in a 250-μL microvial (a limited-volume HPLC vial insert) with 10 μL of furfural reagent.

(3) *N,N-Dimethyl-4-nitrosoaniline test*. In the *N,N*-dimethyl-4-nitrosoaniline test, 1 drop of a 10% *N*,*N*-dimethyl-4-nitrosoaniline in acetic acid (w/v) solution was added to the color plate depression. The plate was heated at 105 °C for 5 min and cooled, and then, a drop of glacial acetic acid was added to the dried residue to visualize the product.

(4) 4-(*Dimethylamino*)*benzaldehyde test*. In this test, 1 drop of a saturated benzene solution of 4-(dimethylamino)benzaldehyde was applied to a filter paper. The benzene was evaporated in a hood at room temperature leaving the dry reagent spot on the filter. Samples or standards were added directly to the 4-(dimethylamino)benzaldehyde reagent spot. Color development was aided by exposure to heat (in a 105 °C oven) and/or acid.

(5) *Chloranil test*. For this test, 1 drop of a saturated chloranil solution in 1,4-dioxane was added to an aryl amine test analyte contained in a color plate depression.

(6) *Pentacyanoaquoferriate test*. This test involved the sequential addition of 3 drops of aqueous 5% (w/v) hydroxyl amine solution, 1 drop of 2% (w/v) aqueous sodium nitroferricyanide(III) dihydrate, and 0.5 drop of 1-M sodium hydroxide to a sample contained in a color plate well.

(7) *Fluorescamine test*. Initial tests with fluorescamine were performed on filters that were dipped in a fluorescamine solution (10 mg in 100-mL acetone) and allowed to dry. 3,4-Dimethylaniline and triethylenediamine standards were spotted on the reagent-loaded filters, and the formation of fluorescent products allowed to proceed for 5 min before visualizing under a handheld 4-watt 365-nm light source (model UVGL-25, UVP, Upland, CA, USA).

2.5. Color tests on C.I. Solvent Red 164 oxidation products

Several of the color spot tests were evaluated on the residue left after evaporation of 100 μL of the impinger solution produced during quartz tube oxidation of C.I. Solvent Red 164. The color tests evaluated included the 4-(dimethylamino)benzaldehyde, glutaconic aldehyde, furfural, pentacyanoaquoferriate, and chloranil tests.

2.6. Color tests on diesel exhaust

A Pramac (Casole d'Elsa, Italy) Model 04604 diesel generator powered by a Yanmar L100-10 HP diesel engine (Adairsville, GA, USA) was used for these studies. The single-cylinder, 0.435-L displacement engine was operated at 3680 rpm, as measured with a Diesel Tiny-Tach tachometer (M&D Small Engine, Bedford, IN, USA). Trials were always conducted using a warm engine. The generator was equipped with a second fuel tank to allow rapid switching between the experimental (dyed) and the control (non-dyed) diesel fuels. Fuel lines were thoroughly flushed after switching fuel sources to eliminate carryover.

Exhaust samples were collected on filter papers for aryl amine analysis using either the 4-(dimethylamino)benzaldehyde or the fluorescamine tests. Exhaust collection used a filter holder fashioned from heavy-duty screen (0.52-mm diameter wire woven with a 4.72-mm wire spacing). This holder configuration allowed the filter to be sandwiched between two screens and positioned at a 45° angle at 9 in. relative to the exhaust port during collection. The holder also prevented exposure of the operator to the heated exhaust and allowed easy access to the filter for application of acidic solutions to improve collection efficiency and color development.

Initial tests with 4-(dimethylamino)benzaldehyde used pre-spotted filters that were moistening with an acetic acid solution (25%, v/v) before exposure to the exhaust. The filter was re-moistened with acid spray at 30-s intervals thereafter during the 3-min exhaust collection period. Color differences were evaluated immediately after exposure. Initial exhaust tests that used fluorescamine collected the exhaust directly on a reagent-free filter paper that was moistened with acetic acid solution. After collection, the dried filter was immediately taken to the laboratory where it was sprayed with an acetone solution of fluorescamine and dried for 5 min. The fluorescent products then were viewed under a 365-nm light source.

Additional tests were performed on filters that were treated with non-volatile acids. For tests using 4-(dimethylamino)benzaldehyde, the reagent was spotted on the filters before treating with succinic, phosphoric, or sulfuric acids. In all cases, the filters were sprayed with a 0.135-M aqueous solution until moist. The filters were air dried and then stored in a desiccator until use.

3. Results and discussion

3.1. GC/MS analysis of C.I. Solvent Red 164 oxidation pyrolysis products

To approximate engine combustion, C.I. Solvent Red 164 was heated in a quartz tube in the presence of an air flow. The thermal decomposition products were collected in an impinger for GC/MS analysis. The temperature for this study was based on the average diesel exhaust temperature for gases exiting the cylinder. Diesel exhaust temperatures range from 540 to 650 °C depending on engine design, work load, and the fuel/air mixing ratio [9]. This temperature is significantly lower than peak combustion temperatures that can exceed ∼1600 °C [10,11]. However, peak temperatures are reached only for short periods at cylinder locations removed from the surfaces, with temperatures significantly lower adjacent to the cylinder walls (i.e., ∼180 °C) [12]. Based on this information, a temperature of 550 °C was chosen for use in the C.I. Solvent Red 164 oxidation/pyrolysis studies.

GC/MS analysis of the quartz tube oxidation products of C.I. Solvent Red 164 showed a number of characteristic products. The total ion current chromatogram of the impinger solution is shown in Fig. 2. A mass spectral library search of the separated components in Fig. 2 resulted in the tentative identifications shown in Table 1. A spectral match quality of over 89 represents an identification that has a high probability of being correct. Among the prominent products that give excellent spectral matches are a variety of alkyl aryl amines. Several of these have been tentatively identified as being 2-methylaniline (*o*-toluidine) that elutes at 5.745 min and 3,4-dimethylaniline with a retention time of 6.403 min. Several additional pyrolysis mixture components give high-quality mass spectral library matches with azo compounds. These tentatively identified compounds include 2,2′-dimethylazobenzene (retention of 12.091 min) and 2-methyl-4-[(2-methylphenyl)azo]-benzamine (retention of 16.119 min). It is likely that the azo products are responsible for the orange coloration of the impinger solution.

The alkyl aryl amine decomposition products observed in the impinger solution arise from reductive cleavage of the azo bonds contained in C.I. Solvent Red 164. The azo decomposition products

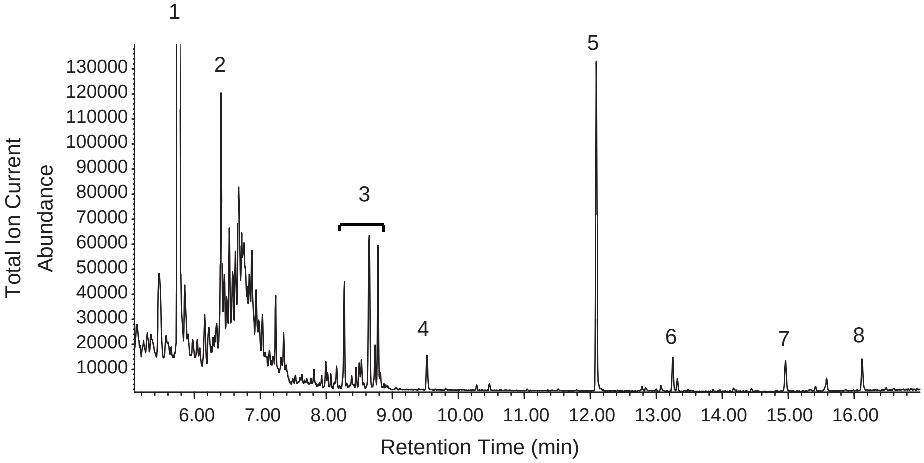


Fig. 2. GC/MS total ion current chromatogram produced on a 30 m × 250 μm i.d. Rtx-5HT column. Peak numbers refer to the numbers in Table 1. Separation conditions are given in the text in Section 2.3.

are formed by cleavage of only one of the two azo bonds contained in the dye molecule. A number of studies that examined pyrolysis of aromatic azo compounds have observed similar aryl amine decomposition products [13,14]. The pyrolysis temperature used in this study was within the literature temperature range where production of aniline derivatives and monoazo products has been observed from decomposition of azo dyes. One study describes bond cleavage mechanisms for aromatic azo compounds and further discusses the thermodynamics associated with the pyrolysis routes [15]. The postulated thermodynamically favored process involves the formation of a bi-radical intermediate from the azo bond, with subsequent successive hydrogen abstraction steps to sequentially form the hydrazo radical and then the hydrazo compounds, respectively, followed by cleavage of the nitrogen–nitrogen bond to form aromatic amines. The free radical species formed during the process can undergo a number of side reactions, including combination reactions to form a variety of products.

Several lower quality spectral library matches, along with a number of unknown aromatic compounds, also were observed in the chromatogram. The structures shown in Table 1 with spectral quality matches below 80 are provided only to illustrate structures that could be similar to the actual compounds. A series of compounds that elute with retention times ranging from 8.10 to 8.79 min all have mass spectra that feature a m/z 147 base ion and a possible molecular ion at m/z 204. Table 1 gives a possible structure for the compound eluting at 8.783 min; however, this represents only one structural possibility for the oxidation products that might be present. A compound that elutes at 9.521 min has a spectrum that is suggestive of yet another oxidation product. The pair of peaks that elute near 13.252 min feature base ions at m/z 105, possible molecular ions at m/z 224, and general spectral features that suggest diphenylpentane isomers. Finally, the unknown compound eluting around 14.957 min features ions at m/z of 237, 210, 118, and a base ion at m/z 91.

The quartz tube oxidation experiment on C.I. Solvent Red 164 clearly indicates that characteristic aryl amine remnants can survive simulated oxidation at diesel engine exhaust temperatures. Obviously, the oxidation/pyrolysis process that occurs in the quartz tube used in our test is only a crude approximation of the combustion processes in a diesel engine cylinder. Engine combustion is a significantly more complex phenomenon because of multiple additional factors, such as the presence of the high pressures, hot catalytic surfaces, and numerous reactive intermediates. This higher complexity might result in lower alkyl aryl amine survivability, or different chemical compositions in diesel exhaust compared to the products from our quartz tube studies. The quartz tube oxidation studies, however, give a simplified, more-easily interpreted approximation that provides solid evidence that alkyl aryl amine dye remnants may be present in exhaust from engines burning azo-dye-tagged fuel. Based on these results, further field test development focused on detection of aryl amines in exhaust as unique molecular remnants that identify the use of dyed fuel. Specifically, 3,4-dimethylaniline was used in our studies because this compound was identified as one of the actual C.I. Solvent Red 164 dye cleavage products present in the impinger solution.

3.2. Color tests

The results of the seven color tests [5,6] investigated are summarized in Table 2. Information in Table 2 includes the color of the reagent blanks, the color of the product formed upon reaction with 3,4-dimethylaniline, and the approximate detection limit. The test results are briefly discussed below:

(1) *Glutaconic aldehyde test*. The reagent blank was a yellow-orange color that could be distinguished easily from the red-orange product formed with 3,4-dimethylaniline. This test was readily capable of visualizing 50 μg of alkyl aryl amine.

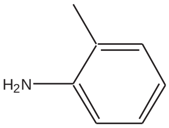
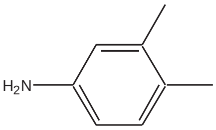
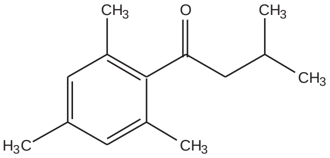
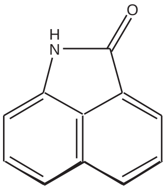
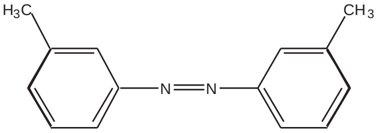
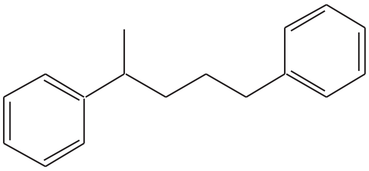
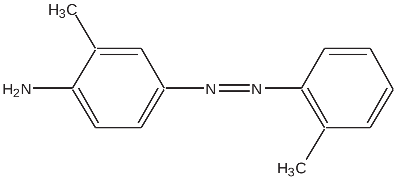
(2) *Furfural test*. The furfural test gave a clear reagent blank and a magenta condensation product with aryl amines. On color plates, this test can easily visualize 50 μg of 3,4-dimethylaniline; however, as with other tests that use reagents dissolved in glacial acetic acid, the surface tension tends to make the reaction mixture diffuse along the color plate surface rather than collect at the bottom of the plate depression. This behavior limits the sensitivity of the furfural test. To overcome this problem, the test was conducted in a micro-vial. The small volume contained in the micro-vial allowed 4 μg of 3,4-dimethylaniline to be detected easily.

(3) *N,N-Dimethyl-4-nitrosoaniline test*. The acetic acid solvent caused dispersal of the reagent along the plate surface. Consequently, this test was not very sensitive, requiring over 50 μg of 3,4-dimethylaniline to produce a maroon condensation product that was easily distinguishable from the yellow-green reagent blank.

(4) 4-(*Dimethylamino*)*benzaldehyde test*. 4-(Dimethylamino) benzaldehyde spots on filter paper (a reagent blank) remained colorless after acid fuming or exposure to heat. Addition of aryl amines to the reagent spot resulted in a bright, canary-yellow color. This test is sensitive, being able to detect 4 μg

Table 1

Analyte peak identifications (corresponding to Fig. 1) showing retention times, significant mass spectral ions, along with tentative identifications and structures for the analysis of Solvent Red 164 quartz tube oxidation products.

Analyte peak identification	Retention time (min)	Significant m/z (relative intensity)	Tentative identifications	Library match quality[a]	Comments
1	5.745	107(75), 106(100), 89(7), 79(12), 77(17)		97	2-Methylaniline
2	6.403	121(100), 120(88), 106(84), 91(15), 83(20), 69(19)		96	3,4-Dimethylaniline
3	8.10 to 8.79; all spectra are related; spectrum for representative peak at 8.783 min is shown	204(6), 147(100), 133(12), 119(17), 107(8), 91(6), 77(3)		72	Compounds contain a prominent m/z 147 and 204 ions. Low quality match is only suggestive of a possible structure
4	9.521	169(100), 141(20), 114(39), 88(12)		54	Benz[cd]indol-2(1H)-one
5	12.091	210(26), 165(6), 119(8), 91(100), 65(24)		90	2,2′-Dimethylazobenzene
6	13.252	224(41), 165(8), 105(100), 91(43), 79(19), 77(23), 65(17)		67	1,4-Diphenylpentane
7	14.957	237(55), 210(61), 118(79), 107(15), 91(100), 77(17), 65(40)	Unknown structure	–	Unknown
8	16.119	225(30), 134(13), 107(27), 106(100), 91(25), 79(19), 77(21), 65(13)		97	2-Methyl-4-[(2-methylphenyl)azo]-benzamine

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[a] Library match qualities of above 90 are considered excellent matches with the library reference spectra. Qualities below 50 indicate substantial differences between experimental and reference spectra exist.

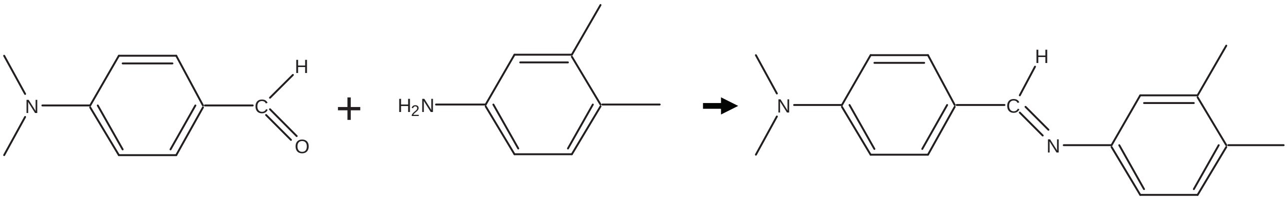

Fig. 3. Reaction between 3,4-dimethylaniline and the 4-(dimethylamino)benzaldehyde to produce the bright yellow condensation product shown. This color test is conveniently performed on filter paper.

Table 2
Visual and fluorescent spot tests used to detect alky aryl amines. Colors for the reagent blanks and reactions with 3,4-dimethyl aniline are given along with the approximate detection limits.

Color test	Format	Analyte visualization	Reagent blank	Format	Approximate detection limit	Comments
Visual tests						
Glutaconic aldehyde	Color plate	Red orange	Yellow-orange	Color plate	<50 µg	
Furfural	Microtube	Magenta	Clear	Microtube	4 µg	
N,*N*-Dimethyl-4-nitrosoaniline	Color plate	Maroon	Yellow-green	Color plate	>50 µg	Toxic reagent
4-(Dimethylamino)-benzaldehyde	Filter paper	Canary Yellow	Colorless	Filter paper	4.0 µg	Attractive format for field test
Chloranil	Color plate	Blue	Light yellow	Color plate	4.0 µg	
Pentacyanoaquoferriate	Color plate	Maroon	Orange	Color plate	<50 µg	Only product that is water soluble; color is not stable
Fluorescence test						
Fluorescamine	Filter paper	Blue or green fluorescence under 360 nm illumination	Non-fluorescent	Filter paper	15 ng	

of 3,4-dimethylaniline. The chemical reaction between 4-(dimethylamino)benzaldehyde and 3,4-dimethylaniline to produce the yellow condensation product [5] is shown in Fig. 3. The unique filter paper format is convenient for the development of a field test.

(5) *Chloranil test*. A dark blue color immediately formed with aryl amines at ambient temperature. The light yellow reagent blank was easily distinguished from the dark blue condensation product. This test can easily detect 4 µg of 3,4-dimethylaniline.

(6) *Pentacyanoaquoferriate test*. The nitroprusside reagent $[(NC)_5Fe(III)NO]^{2-}$ forms a pentacyanoaquoferriate $[(NC)_5Fe(II)H_2O]^{3-}$ complex when exposed to hydroxylamine in the presence of base [16]. This is thought to occur through an intermediate formed by the addition of hydroxylamine to the nitrosyl ligand, followed by decomposition of the adduct resulting in the formation of nitrous oxide and water. The Fe(III) is reduced to Fe(II) during this process. Once formed, nucleophiles, including aryl amines, react with the pentacyanoaquoferriate complex by displacing the coordinated water to give colored complexes [5,17].

The nitroprusside reagent blank was orange, but reaction with 3,4-dimethylaniline yielded a dark-maroon, water-soluble complex that was readily detectable at 50 µg. Our studies showed a serious disadvantage of this test was that the colored ferropentacyanamine complex was not stable and began to fade within 5 min. Oxidation of the Fe(II) core or competitive coordination with ligands like carbon monoxide are processes that could account for the observed limited complex stability. Reaction of oxygen with ferropentacyanamines has been reported to be slow in the presence of base [18]. Regardless, further development efforts should focus on testing under an inert atmosphere to enhance the color permanence.

(7) *Fluorescamine test*. Alkyl amines (pKa ∼ 10–11) are more reactive compared to aryl amines (pKa = 5) because the higher basicity of the nitrogen [19]. Many reagents used to visualize alkyl amines do not react with the less reactive aryl amines. Fluorescamine is a non-fluorescent reagent that forms fluorescent products with amines including aryl amines and was, therefore, of interest for this study [7]. Detection of fluorescent adducts is expected to be approximately 100–1000 times more sensitive compared to detection of visible color spots [20]. Analysis of standards gave a green fluorescent product with 3,4-dimethylaniline, whereas dietheylenetriamine gave a product that emitted blue fluorescence. The longer emission wavelength for the 3,4-dimethylaniline product was likely to be the result of the more extensive resonance delocalization in the product compared to the diethylenetriamine product. The detection limit for this visualization was approximately 15 ng of amine.

3.3. *Color tests on the C.I. Solvent Red 164 pyrolysis products*

A number of color spot tests were evaluated using the residue left after evaporation of methylene chloride from 100 µL of the quartz tube oxidation impinger solution. This is a more realistic test than evaluating pure standards because the more chemically complex mixture may contain matrix components that interfere with the analysis. The 10 mg of C.I. Solvent Red 164 used in the quartz tube oxidation studies corresponds to the quantity of dye contained in roughly 0.91 L of diesel fuel. Because the decomposition products were captured in a 20-mL volume of solvent, the amount of residue contained in a 100-µL aliquot of impinger solution corresponds to the pyrolysis products expected from 4.6 mL of dyed diesel fuel. For perspective, the fuel consumption of the warm diesel generator was determined to be 12.72 mL/min (i.e., 4.6 mL of fuel every 22 s).

Of the tests conducted with the impinger residue, 4-(dimethylamino)benzaldehyde and glutaconic aldehyde spot tests gave unambiguous, positive results for aryl amines with clear differences between the reagent blanks and the experimental sample. The furfural spot test also was evaluated and gave a color difference between the experimental and reagent blank; however, further testing revealed that the color formed was not distinguishable from the residue alone dissolved in glacial acetic acid. In this case, the orange-red pyrolysis residue could not be distinguished from the furfural test performed on the residue. Therefore, we felt that the furfural test, despite its high sensitivity, would not be appropriate for this particular application.

The pentacyanoaquoferriate and chloranil tests gave negative results when performed on the C.I. Solvent Red 164 residue. For the chloranil test, the negative result was surprising given the high sensitivity of the test when pure standards were evaluated (see Table 2). Based on the results discussed above, further development of the pentacyanoaquoferriate and chloranil tests was suspended so we could focus our efforts on the two tests that showed the highest degree of promise for the dye decomposition products.

3.4. *Microplate reader*

A Biotek Synergy H4 Multi-Mode microplate reader (Winooski, VT) was used to demonstrate the quantitative potential for the 4-(dimethylamino)benzaldehyde test. Standards for a calibration curve were prepared by adding 3,4-dimethylaniline to Ehrlich's reagent. The resulting condensation product gave an absorption maximum at 440 nm; a wavelength that was used for subsequent absorbance measurements. A standard curve was obtained that ranged from 0.8 to 8.0 mg/L and gave a linear regression equation of $y = 0.2363x + 0.0039$ ($r^2 = 1.0$). Evaluation was performed by applying 1.6-μg quantities of 3,4-dimethylaniline directly on a spot of 4-(dimethylamino)benzaldehyde reagent that had been applied to filter paper. The spots then were manually cut out and extracted with 1.0 mL of glacial acetic acid:methanol:water (5:3:2, v/v) mixture [i.e., Ehrlich's reagent without 4-(dimethylamino)benzaldehyde], and the absorbance was determined by the microplate reader. The color-forming reaction was not reagent limited within this concentration range even though the filter extract contained a slightly lower amount of the 4-(dimethylamino)benzaldehyde reagent (4.8 ± 0.3 mg/mL) compared to Ehrlich's reagent (7.5 mg/mL). Analysis of the extracted filter paper spots gave 97% recovery for 3,4-dimethylaniline. Although the overall goals of this study were to develop a qualitative test for detecting dye remnants in diesel-engine exhaust, this experiment clearly highlights the potential for providing sensitive quantitative determinations.

3.5. *Acid filter treatments to enhance exhaust sampling and color development*

Gas-phase capture of alkyl aryl amines and color development using 4-(dimethylamino)benzaldehyde reagent is enhanced by the presence of acid [5]. One approach is to spray the filter before and intermittently during exhaust sampling with an aqueous acetic acid spray. This provides a liquid phase and acidic environment for capture and reaction on the filter. One consideration is whether residual acid left after evaporation (if any) would interfere with the fluorescamine reaction. Optimal pH for the reaction of fluorescamine with primary amines is known to be in the range of 8–9.5 [21]. However, some researchers have shown that aryl amines form fluorescent products at lower pH values (pH = ~5.8) without a significant reduction in fluorescence intensity [22]. To test whether fluorescence was inhibited by the presence of residual acid, clean filters spotted with 3,4-dimethylaniline were moistened with 25% acetic acid spray, dried, and then sprayed with fluorescamine reagent. The results showed that the aryl amine standard could be detected with approximately the same detection limit as filters that did not receive the acetic acid treatment. From these results, it does not appear that the acetic acid treatment used during exhaust collection would interfere with the detection of aryl amines using fluorescamine visualization.

Further laboratory tests evaluated 4-(dimethylamino)benzaldehyde-spotted filters that were made acidic by spraying with non-volatile acids [23]. Filters were sprayed with a 0.135-M aqueous solutions of sulfuric (pKa = −3), phosphoric (pKa = 2.14), or succinic (pKa = 4.12) acids, and then allowed to dry leaving the non-volatile acid residue on the filter. For reference, acetic acid (pKa = 4.76) is a slightly weaker acid than succinic acid. Because these acids are not volatile, the filters should not require in-field spray applications. The disadvantage of this approach is that a liquid phase is absent during collection (in contrast to the aqueous acetic acid treatment) so collection efficiency may be dramatically reduced as a consequence. Laboratory tests involved applying the 3,4-dimethylaniline test analyte, noting any visible color differences under room lights, heating at 105 °C in an oven, and then noting the fluorescence under 365-nm ultraviolet illumination.

The laboratory tests found the stronger acids (sulfuric and phosphoric) resulted in distinctly yellow 4-(dimethylamino)benzaldehyde control spots that were visually difficult to differentiate from the yellow-orange color resulting from application of a 3,4-dimethylaniline standard. On the other hand, succininc acid gave a nice differential color between the clear reagent control and the yellow experimental spots. When the filters were heated and then viewed in 365-nm light, very different results were obtained. The stronger acids resulted in intense orange fluorescent spots for the experimental samples, whereas the control reagent spots were non-fluorescent. Succininc acid resulted in weak orange fluorescence for the test analyte, and acetic acid treatment gave light green-yellow fluorescence for both the control reagent spots and experimental samples. Clearly the fluorescent tests using strong non-volatile acid-treated filters have potential for successful application as field tests. Overall, the preliminary laboratory experiments showed results that correlated with the acid strength. The weaker acids (i.e., acetic and succinic acid) resulted in control reagent spots that exhibited minimal color, while the yellow aryl amine condensation product was easily visualized. For fluorescence visualization, acids stronger than succinic acid are needed to form an orange fluorescent product.

Additional tests were conducted by spotting phosphoric acid-treated, reagent-free filters with 3,4-dimethylaniline, and then spraying with fluorescamine immediately or after neutralizing with a 0.1-M phosphate buffer spray at pH = 7.5 and allowing the filters to dry. Although neutralization resulted in a slightly more intense fluorescence, direct spraying of the acid-treated filter with fluorescamine also was highly effective. The direct treatment (without neutralization) was implemented for the field test described below because it was simpler, reasonably sensitive (easily able to detect <50 ng of aryl amine) and was potentially more selective because primary amines were less likely to be detected on the acidic filters [22].

3.6. *Exhaust analysis*

3.6.1. *4-(Dimethylamino)benzaldehyde test*

During exposure to the exhaust, heat from the exhaust, and the acid conditions, both facilitated color development when the 4-(dimethylamino)benzaldehyde reagent was used. Temperatures measured during collection of an exhaust sample ranged from 120 to 125 °C at the diesel generator exhaust port and was 56 °C 9 in. away from the exhaust port where collection filters were positioned. During an initial trial, duplicate control and experimental filters were exposed. Later in the project, the test was independently repeated. Photographs that document the results for this test are shown in Fig. 4. The filters in this figure show duplicate 4-(dimethylamino)benzaldehyde test filters that had been exposed to exhaust from an engine burning dyed diesel fuel (Fig. 4, top) compared to control filters exposed to exhaust from an engine burning non-dyed fuel (Fig. 4, bottom). The differences were striking; the compounds in exhaust from combustion of dyed fuel produced much more intense yellow spots than compounds in exhaust from the non-dyed fuel. The results for the second independent trial were comparable to the first trial and, therefore, served to verify the initial findings. The results definitively show that the 4-(dimethylamino)benzaldehyde test using and acetic acid-wetted filters can be used to identify engines burning dyed fuel and to distinguish those engines from those burning non-dyed fuel.

These results suggest the developed color spot test could readily be applicable to testing vehicles under realistic operating conditions. During the 3-min field trials, the generator test engine consumed 424 μg of C.I. Solvent Red 164. Typical diesel pickup trucks and eighteen-wheeler tractors contain engines with displacements of 6.9 L and 16 L, respectively. These displacements are up to 30 times the displacement of the 0.435 L test engine used in this study. Clearly, the larger vehicle engines consume more fuel with the consequence that an equivalent amount of C.I. Solvent Red 164 would be consumed in a shorter period of time. Using a

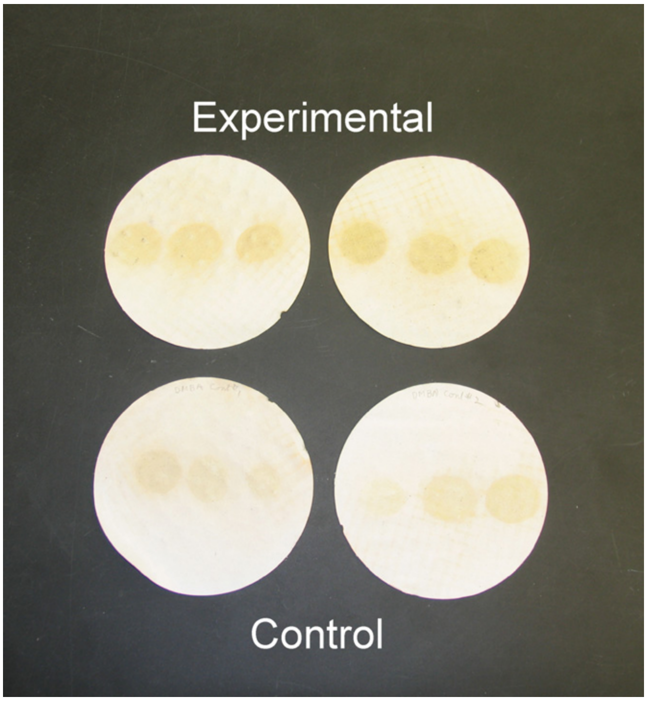

Fig. 4. Proof-of-principle experiment showing the 4-(dimethylamino) benzaldehyde test when applied to diesel exhaust from an engine burning dyed fuel (top) compared to non-dyed fuel (bottom).

conservative estimate of an order-of-magnitude higher fuel consumption in vehicles compared to the test engine, color spot tests should be able to be completed within 20 s. Such a short sampling period would be convenient for rapid screening of vehicles. Although the proof-of-principle test gives irrefutable results, further development would be desirable before being implemented by field personnel. Repetitive spraying with acetic acid works well; however, the procedure is labor intensive.

Field tests that used non-volatile acidic filters containing 4-(dimethylamino)benzaldehyde yielded usable results only for filters treated with phosphoric acid. These filters showed a visible difference in color between the experimental and control trials, with exposure to exhaust from dyed fuel resulting in a darker, more-orange spot than the yellow spot that resulted from exposure to exhaust from control fuel. Although the control and experimental filters were clearly distinguishable, the color differential was not pronounced. Other field trial tests using either visual or fluorescence detection gave control and experimental results that were difficult to distinguish. Overall, despite the distinct operational advantages associated with avoiding reapplication of acid during the field test, it appears that the trials using acetic acid during collection produced the best experimental results to date. We speculate that this may be the result from more efficient capture and reaction with 4-(dimethylamino)benzaldehyde on the filter when a liquid phase is present. Future tests could investigate collection on filters containing non-volatile acids combined with intermittent or continuous infusion of water spray to provide a liquid capture/reaction phase on the filter. This approach would still avoid safety issues and the objectionable odor associated with field application of acetic acid.

Additional development should also focus on making the test easy to interpret. For example, although the color differences between controls and experimental exhaust exposures were pronounced during the acetic acid spray trials, the control filter spots did have a slight light yellow coloration. If control tests were not conducted side-by-side with experimental exposures, interpretation of the results would have been more difficult. Visible comparison to pre-prepared color strips that define threshold color values would help to unambiguously distinguish between positive and negative test results.

The field test developed is intended to detect the use of dyed fuel in real time. However, given the semi-volatile nature of some of the larger-molecular-weight alky aryl amines, these combustion products may partially condense in the exhaust train, only to be released in the exhaust gases at a slower rate. This could make the test capable of not only detecting the current use of dyed diesel fuel, but also its recent use. Finally, it would be desirable to further analyze the exposed filters for: (1) specific reaction products as a way of validating the method and/or (2) specific cases that require more scientific rigor. This could be accomplished by analyzing the yellow spots for the presence of specific condensation products formed between 4-(dimethylamino)benzaldehyde and the characteristic aryl amines produced by dye combustion. A number of laboratory-based analytical techniques, including direct analysis in real time-mass spectrometry (DART-MS) [24] of the spots or solvent extraction followed by liquid chromatography (LC)/MS or GC/MS, could provide this capability.

3.6.2. Fluorescamine test

Results from the exhaust experiment using acetic acid spray showed that filters exposed to dye-containing fuel had a discernibly higher intensity of green fluorescence than filters from the control. We attempted to capture the difference using digital photography; however, the subtle differences were not pronounced enough to be evident in the photographic images. We believe these results indicate that further development of this test is warranted. Collections on phosphoric acid-treated filters also were performed. In this case we did not observe fluorescence on either the controls or the experimental samples. One possible explanation is that capture may be inefficient on the dry acidic filters relative to the aqueous acetic acid spray approach.

4. Conclusions

In summary, tax-free fuel is indentified in the United States by the presence of an azo dye, C.I. Solvent Red 164, that is added at a concentration of about 11.1 mg/L [2,3]. A field screening test performed on diesel-engine exhaust to detect the use of dyed fuel would be distinctly advantageous over the currently used visual inspection of the fuel for the characteristic red color of tax-free fuel. Work described in this study started with the hypothesis that certain characteristic dye remnants might survive engine combustion and be present in the exhaust. Initial studies confirmed the potential for the presence of dye remnants by examining the pyrolysis of C.I. Solvent Red 164 in the presence of oxygen. GC/MS analysis of the products revealed the presence of alkyl aryl amines as well as several azo compounds. Based on these results, a number of traditional micro-analytical spot tests were evaluated for their potential to detect alkyl aryl amine standards and residues contained in a C.I. Solvent Red 164 oxidation product mixture. This process allowed selection of the most promising micro-analytical color spot tests based on performance and compatibility with producing a cost-effective, easy-to-use, and unambiguous color test that can be implemented in the field by fuel compliance specialists.

Of the tests evaluated, the 4-(dimethylamino)benzaldehyde test was found to have the most potential for field application. Two independent trials exposed 4-(dimethylamino)benzaldehyde-spotted filters in duplicate to diesel exhaust from engines burning either non-dyed fuel (controls) or dyed fuel (experimental). Intermittent applications of acetic acid were used during exhaust collection to facilitate capture and color development.

Duplicates from both independent trials showed exposure to experimental exhaust produced a more intense yellow spot test when compared to the controls. The differences were distinct and easy to recognize, thus providing definitive proof that the 4-(dimethylamino)benzaldehyde test can effectively identify engines burning diesel fuel dyed with C.I. Solvent Red 164.

Several additional methodologies showed potential that might warrant further development. Collection on phosphoric acid-treated filter papers gave experimental results that could be clearly distinguished from the controls. Fluorescamine treatment following collection on acetic acid-treated filters also gave subtly different controls and experimental trials. However, in both cases the color differentials between controls and experimental trials were not as pronounced as for the 4-(dimethylamino)benzaldehyde test performed with acetic acid-facilitated collection.

Various countries around the world use azo dyes as fuel markers. For example, Canada uses a red/purple dye and C.I. Solvent Yellow 124, whereas the European Union uses C.I. Solvent Yellow 124 (also known as Euromarker) [25]. The tests described in this paper should be directly applicable, or readily adaptable, to exhaust analysis for these and other azo dyes as well, and could offer compliance officials a viable alternative with all associated advantages for detecting the use of dyed fuels by analyzing the exhaust rather than by direct visual inspection of the fuel.

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