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Gradient reversed-phase liquid chromatographic-electrospray ionization mass spectrometric method for the comparison of smokeless powders

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Abstract

A gradient reversed-phase liquid chromatographic-electrospray ionization mass spectrometric (LC-ESIMS) method was developed to determine compositional variation in the organic additives of smokeless powders. The method was optimized for the separation and detection of selected powder constituents, including diphenylamine, along with isomers of its nitroso and nitro derivatives, centralite I and II, in addition to dialkylphthalate acid esters. A series of commercially available smokeless powders was prepared by organic liquid extraction and characterized using the LC-ESIMS method. The results demonstrate the differentiation of smokeless powders by their additive profile.

Keywords: Explosives; Smokeless powders; Diphenylamine; Nitrosodiphenylamine; Methyl centralite; Ethyl centralite; Dimethylphthalate; Diethylphthalate; Dibutylphthalate

1. Introduction

Smokeless powders are manufactured for optimum propellant performance for small arms ammunition. Due to different propellant applications and reformulation, there is a wide range of compositional differences between commercially available smokeless powders. These unique differences in smokeless powder composition allow for the determination of a distinguishable chemical profile [1–4]. Since smokeless powders can be purchased for reloading ammunition, a potential problem for forensic investigators is the use of these propellants in improvised explosive devices (IEDs) such as pipe bombs. Following a bombing incident involving smokeless powders, a portion of unburned powder may be recovered from the crime scene. The organic constituents detected in the unburned powder or its residue can be used to characterize the questioned powder [5–10].

The forensic analysis of smokeless powders is carried out by identifying and quantifying the organic components using instrumental techniques. Gas chromatography (GC) and liquid chromatog-

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raphy (LC) have been used with various detection modes for the analysis of organic additives in smokeless powders [1–14]. While GC methods are widely used, a major disadvantage is the thermal degradation of certain nitrated components. LC methods can also be used. Due to the wide range of polarity of the components and difficulty in separating geometrical isomers, isocratic normal-phase and reversed-phase LC methods are limited to the analysis of certain organic additives [1–7].

Mass spectrometry (MS) has been employed for the detection of the organic constituents in smokeless powders [8–12]. For example, Martz and Lasswell used GC–MS results and morphological properties to differentiate smokeless powders [8]. Reversed-phase LC methods with MS detection have also been employed. LC-thermospray ionization MS was used to detect diphenylamine (DPA) and nitroglycerine in residue from pipe bombs loaded with smokeless powder [9,10]. LC-electrospray ionization mass spectrometry (ESIMS) was used for the analysis of methyl centralite (MC) in a single smokeless powder from gunshot residue (GSR) deposited on human hands [11]. ESIMS was employed without chromatographic separation to quantify DPA and its nitrated derivatives from GSR [12]. Despite the success in demonstrating the detection of organic smokeless powder additives from GSR, the previous ESIMS studies were limited in the number of additives detected and in the variety of smokeless powders tested [11,12].

Disadvantages in particular analysis techniques, such as thermal degradation in GC–MS and the limited number of components determined by LC methods, has led to the development of alternative methods. A gradient reversed-phase LC method (LC–UV, photodiode array) has been developed in this laboratory for the analysis of the organic constituents in smokeless powders [13]. In the present study, this gradient reversed-phase LC method was modified to facilitate detection ESIMS in the positive ion mode. The gradient reversed-phase LC-ESIMS method was developed to establish a more comprehensive ESIMS method of determination for organic powder constituents and to differentiate unburned smokeless powders by identifying and quantifying particular additives.

2. Experimental

2.1. Chemicals

Ammonium acetate and HPLC grade solvents: methylene chloride and methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). Milli-Q purified water was used throughout the experimental procedures. Analytical standards: *N*-nitrosodiphenylamine (*N*sDPA) (Fluka, Milwaukee, WI), diphenylamine (DPA), 4-nitrosodiphenylamine (4sDPA), 2-nitrodiphenylamine (2NDPA), 4-nitrodiphenylamine (4NDPA), 4-4′-dinitrodiphenylamine (4-4′-DNDPA), methyl centralite (MC), ethyl centralite (EC), dimethylphthalate (DMP), diethylphthalate (DEP), and dibutylphthalate (DBP) (Acros, NJ, USA) were prepared in methanol as 0.5- or 1-mg/ml stock solutions and stored at 4 °C.

2.2. Equipment

2.2.1. LC conditions

A Hewlett-Packard 1100 LC system (Agilent, Palo Alto, CA, USA) controlled by Chemstation software (A.06.03) was used with a Restek Pinnacle octyl column: C_8, 2.1×100 mm, 3-μm particle size, and 120-Å average pore size at room temperature. The LC detection system consisted of a variable wavelength UV detector, at 230 nm, coupled to a Bruker Esquire (Bruker Daltonics, Bremen, Germany) electrospray ionization interface with a quadrupole ion-trap mass spectrometer. The mobile phase consisted of methanol and 1 m*M* aqueous ammonium acetate with a linear gradient of 50–95% methanol in 25 min at 0.25 ml/min. Standard mixtures with concentrations between 0.05 and 100 μg/ml were prepared weekly for validation studies. An autosampler injection volume of 5 μl was used for the LC-ESIMS analysis.

2.2.2. Electrospray ionization mass spectrometry

For ESIMS optimization, analytical standards at 100 μg/ml were infused using a Cole-Parmer Syringe Infusion Pump (Cole-Parmer, Vernon Hills, IL) at 0.05 ml/min. The ESIMS was operated in the positive ion mode, 4.0 kV spray voltage, nitrogen (N_2); 40 p.s.i. nebulizing and 2.5 l/min drying gas,

225 °C capillary temperature, 35 V capillary offset voltage, 25 V skimmer 1, 6 V skimmer 2, 2.4 V octapole, −5 V lens, −60 V lens 2, and data collected from m/z 50 to 500.

2.2.3. Smokeless powder analysis

The smokeless powder samples were selected from various manufacturers including IMR (Plattsburgh, NY, USA), Accurate Arms Company (McEwen, TN, USA), Hogden (Shawnee Mission, KS, USA), and Alliant (Radford, VA, USA). The smokeless powder sample preparation method was developed for GC–MS analysis and adapted for use with LC–UV [8,13]. Individual smokeless powder samples were prepared by extracting 5 mg of the unburned powder with 250 μl methylene chloride overnight. A 20-μl aliquot was removed into a clean vial and evaporated under a stream of nitrogen gas. The samples were reconstituted with 40 μl methanol. An injection volume of 5 μl was used for the quantitative LC-ESIMS analysis.

3. Results and discussion

The reversed-phase gradient separation method developed by Wissinger and McCord for smokeless powder analysis was adapted for ESIMS detection [13]. The ESIMS detection parameters were optimized for the detection of selected powder additives as protonated molecules in the positive ion mode. The parameters were also optimized to reduce the collision-induced dissociation (CID) of dibutylphthalate in the electrospray source [15]. The gradient reversed-phase separation method was modified for ESIMS detection by reducing the column size, flow rate, and adding ammonium acetate to the aqueous portion of the mobile phase. Using the optimal ESIMS parameters, the separation method was used for the determination of diphenylamine and centralite based stabilizers in addition to dialkylphthalate plasticizers.

3.1. Electrospray ionization mass spectrometry optimization

Individual standards containing 100 μg/ml diphenylamine (DPA), 4-nitrosodiphenylamine (4sDPA), 4-nitrodiphenylamine (4NDPA), methyl centralite (MC), ethyl centralite (EC), and dibutylphthalate (DBP) were infused in 50% methanol/1 mM aqueous ammonium acetate to establish maximum electrospray ionization efficiency. The ''in-source'' CID of DBP, a 1,2-benzenedicarboxylic acid ester, M_r 278, was discovered during preliminary experiments by the dominance of the characteristic ions at m/z 149 and m/z 205 in the mass spectrum. ''In-source'' or ''up-front'' CID is a molecular fragmentation process which occurs as ions are transferred from the atmospheric pressure source to the mass analyzer. During ion transfer, the resulting energy from significant pressure and voltage differences between the capillary exit electrode ''nozzle'' and skimmer causes fragmentation [15]. The type of atmospheric pressure ionization interface is an important consideration for diagnosing ''in-source'' CID. In the Bruker-Esquire instrument, the capillary length is ~10 cm. As opposed to interfaces with only a cone or dual capillaries, the pressure differential between the atmospheric source and the reduced pressure region of the skimmers in the Bruker-Esquire ESIMS is dispersed over a large volume. Therefore, the voltage offset, between the capillary exit electrode and skimmer 1, was considered to be the prominent factor contributing to the CID of dibutylphthalate. The nozzle–skimmer voltage offset was reduced from 60 to 10 V to maximize the intensity of protonated molecule of dibutylphthalate, m/z 279, thus reducing ''in-source'' CID.

3.2. LC optimization

The separation method development was performed using the optimized ESIMS parameters for the detection of the selected smokeless powder additives. The reversed-phase gradient method was modified by using a smaller column, lower flow rate, and adding ammonium acetate to the mobile phase to facilitate the electrospray ionization. While Wissinger and McCord [13] employed a column diameter of 4.6 mm and 1.0-ml/min flow rate, the present method was developed for ESIMS detection with a 2.1-mm column. A flow rate was of 0.25 ml/min was used to minimize peak broadening between the

UV flow cell and ESIMS in the coupled detection system. An additional consideration for method development in this study was the particle size of the smaller column. Since the 2.1-mm column contained 3-μm particles, compared to a 5-μm particle size used by Wissinger and McCord, a shorter column length was necessary to achieve efficient separation.

The compounds listed in Table 1 were identified by chromatographic retention and ESIMS spectra as protonated molecules. The retention time for each component was determined using extracted ion chromatograms. The repeatability for retention is shown in Table 1, in which the relative standard deviation for each compound is less than 1%. It should be noted that dimethylphthalate and 4-nitrosodiphenylamine were included in the validation studies as shown in Table 1. These two components were not detected in the selected smokeless powders and were omitted in the subsequent section (Table 2).

3.3. LC-ESIMS method validation

Daily, replicate chromatographic injections of standard mixtures with concentration range from 0.05 to 20 μg/ml were used to determine the accuracy and precision (relative standard deviations, RSD) for extracted ion chromatogram (EIC) peak areas. The repeatability for all standards ranged from 0.4 to 11.6% for intra-assay and 0.6 to 17.3% for inter-assay precision. The method calibration was established using linear regression data for the EIC peak areas of each component over the concentration range of 0.05–20 μg/ml. For example, the calibration plot of peak area (y) versus concentration (C) for 4-4′-dinitrodiphenylamine (4-4′-DNDPA) was $y=4.5\times10^{5}C+4.0\times10^{3}$ with $r^{2}=0.9998$ and a 2-ng detection limit using $S/N=3$. To illustrate the precision of the method, the intra-assay (daily) and inter-assay (day-to-day) variation is shown in Table 1 for the ESIMS response of the 1.0-μg/ml ($n\geq3$) analytical standard. The inter-assay precision is slightly greater than the intra-assay precision as expected with the exception of *N*-nitrosodiphenylamine (*N*sDPA) and methyl centralite (MC). The differences in the variation were not significant, which is beneficial for the simultaneous determination of smokeless powder additives.

3.4. Smokeless powder analysis

The quantitative analysis of 11 different unburned smokeless powders was performed using the LC-ESIMS method. Table 2 shows the percent composition and standard deviation for the components detected. In general, the smokeless powders are distinguishable by the presence or absence of certain compounds. For example, the detection of EC in

Table 1
Retention factor (k), intra- and inter-assay precision (percent relative standard deviation, %RSD) of extracted ion chromatogram (EIC) peak areas the LC-ESIMS method for the determination of smokeless powder additives for the standard sample of 1.0 μg/ml ($n\geq3$)

Compound	$[M+H]^{+}$ m/z	Retention factor		Intra-assay precision (EIC peak area) (within day), %RSD ($n\geq3$)	Inter-assay precision (EIC peak area) (day-to-day), %RSD ($n\geq3$)
		k	%RSD ($n\geq3$)		
Dimethylphthalate	195	7.7	0.7	4.2	5.1
Diethylphthalate	223	10.8	0.5	7.5	7.6
4-Nitrosodiphenylamine	199	11.0	0.4	5.2	8.4
4-4′-Dinitrodiphenylamine	260	12.1	0.4	3.2	6.0
N-Nitrosodiphenylamine	199	12.4	0.3	6.1	5.3
Methyl centralite	241	12.6	0.3	2.3	1.6
4-Nitrodiphenylamine	215	12.9	0.3	2.7	3.8
Diphenylamine	170	13.3	0.4	2.4	3.4
2-Nitrodiphenylamine	215	14.0	0.4	4.1	8.3
Ethyl centralite	269	16.1	0.3	3.1	7.0
Dibutylphthalate	279	16.8	0.3	3.8	7.1

Gradient elution of 50–95% methanol in 25 min and Pinnacle octyl column.

Table 2
Percent composition (%) and standard deviation (SD, $n \geq 3$) of unburned smokeless powder samples determined using methylene chloride extraction and analysis by the LC-ESIMS method with the Pinnacle octyl column and methanol gradient

Compound	m/z	Smokeless powder, percent composition and standard deviation ($n \geq 3$)													
		AL8		H322		H414		H335		2400		RD900		N130	
		%	(SD)	%	(SD)	%	(SD)	%	(SD)	%	(SD)	%	(SD)	%	(SD)
Diethylphthalate	223									0.4	(0.03)				
4-4′-Dinitrodiphenylamine	260														
N-Nitrosodiphenylamine	199	2.0	(0.3)	1.0	(0.08)	0.8	(0.1)	1.3	(0.1)	1.0	(0.3)	0.1	(0.06)	0.4	(0.06)
Methyl centralite	241													0.7	(0.06)
4-Nitrodiphenylamine	215	0.7	(0.1)	0.2	(0.02)	0.1	(0.01)	0.2	(0.02)	0.9	(0.2)				
Diphenylamine	170	2.0	(0.3)	5.2	(0.4)	2.9	(0.3)	3.9	(0.4)	3.5	(0.5)	2.2	(0.8)	3.4	(0.08)
2-Nitrodiphenylamine	215	0.8	(0.1)	0.2	(0.02)	0.4	(0.06)	0.5	(0.1)	0.7	(0.2)	0.1	(0.02)		
Ethyl centralite	269					0.2	(0.03)	0.3	(0.03)	0.2	(0.04)	4.5	(0.8)	5.0	(0.3)
Dibutylphthalate	279	0.4	(0.03)			2.0	(0.1)	2.3	(0.4)	0.5	(0.02)	0.03	(0.01)		

		IMR 4895		IMR 4064		IMR 4350		IMR 4831	
		%	(SD)	%	(SD)	%	(SD)	%	(SD)
Diethylphthalate	223								
4-4′-Dinitrodiphenylamine	260	0.05	(0.01)						
N-Nitrosodiphenylamine	199	0.8	(0.04)	1.0	(0.2)	1.6	(0.2)	1.4	(0.3)
Methyl centralite	241					0.5	(0.01)	0.1	(0.03)
4-Nitrodiphenylamine	215	0.4	(0.04)	0.2	(0.04)	0.2	(0.1)	0.2	(0.05)
Diphenylamine	170	0.6	(0.05)	5.7	(1.3)	3.7	(0.6)	3.3	(0.8)
2-Nitrodiphenylamine	215	0.5	(0.05)	0.3	(0.07)	0.5	(0.1)	0.4	(0.1)
Ethyl centralite	269							0.5	(0.004)
Dibutylphthalate	279	0.04	(0.004)	0.06	(0.01)	0.03	(0.01)	0.4	(0.06)

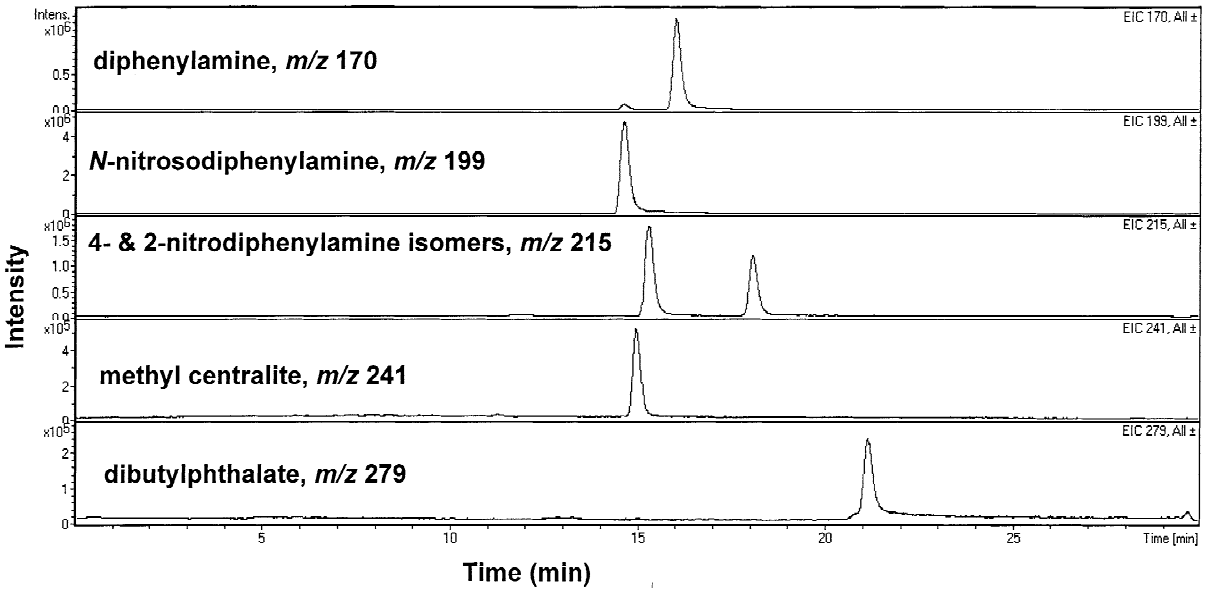

Fig. 1. Extracted ion chromatograms of IMR 4350 smokeless powder illustrating the presence of diphenylamine, *N*-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, methyl centralite, ethyl centralite and dibutylphthalate. LC-ESIMS method using gradient elution, 50–95% methanol in 25 min and the Pinnacle octyl column.

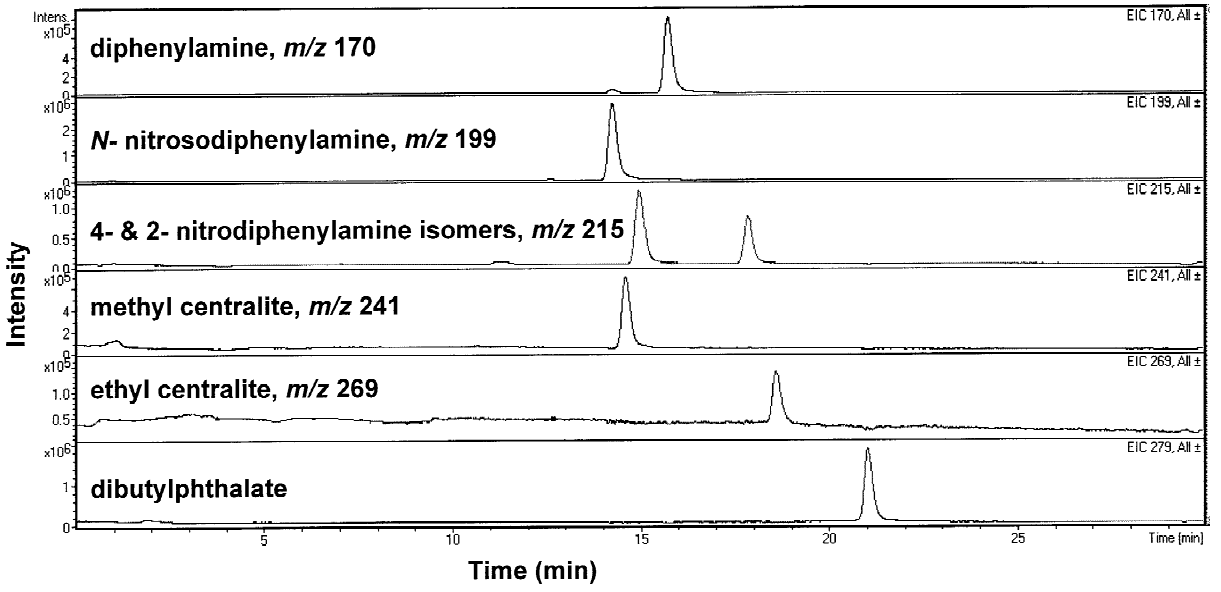

Fig. 2. Extracted ion chromatograms of IMR 4831 smokeless powder, in which diphenylamine, *N*-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, methyl centralite and dibutylphthalate were detected. Conditions as listed in Table 1 and Fig. 1.

IMR 4831 was required to show the difference between IMR 4350 and IMR 4831. The extracted ion chromatograms for these powder samples are shown in Figs. 1 and 2. While both powders contain the compounds DPA, *N*sDPA, 4NDPA, 2-nitrodiphenylamine (2NDPA), MC and DBP, the detection of EC in IMR 4831 illustrates the advantage of the LC-ESIMS analysis method in differentiating smokeless powders. Other powders with similar compositions required more extensive analysis. For example, DPA, *N*sDPA, 4NDPA, 2NDPA, EC and DBP were detected in H414, H335 and IMR 4064. Examination of the percent composition in Table 2 shows that these powders can easily be differentiated by the variation in concentration of specific components. Similar levels of DPA were detected in all three powders but H414 and H335 contain a significantly larger percentage of DBP. H414 and H335 can be distinguished by the difference in the percentage of *N*sDPA and EC detected, for which *t*-tests at the 95% confidence level result in the probability of having equal concentration as 0.01 and 0.02%, respectively.

DPA, *N*sDPA, and one isomer of nitrodiphenylamine were detected in each of the 11 powders. DPA is a stabilizer that is added to smokeless powder to prevent the autocatalytic degradation of NC in the presence of moisture. Nitrous and nitric acids produced during powder aging react with DPA, which results in the formation of nitroso and nitro derivatives of DPA, such as *N*sDPA and 4NDPA [16]. For example, the extracted ion chromatograms of Red Dot 900 are shown in Fig. 3, in which DPA, *N*sDPA, 2NDPA, EC and DBP were detected. Red Dot 900 was the only smokeless powder in which 2NDPA was detected without the 4NDPA isomer as illustrated in Table 2 and Fig. 3. The presence of 2NDPA alone is unique given that the reactivity trend toward nitro derivative formation has been shown to be DPA>4NDPA>2NDPA [14,16]. Another example of the applicability of the LC-ESIMS method was the detection of 4-4′-dinitrodiphenylamine in IMR 4895. The presence of this dinitro isomer indicates that the powder may have been aged more than the others prior to analysis. Fig. 4 shows the extracted ion chromatograms for IMR 4895, in which DPA, *N*sDPA, 4NDPA, 2NDPA, EC,

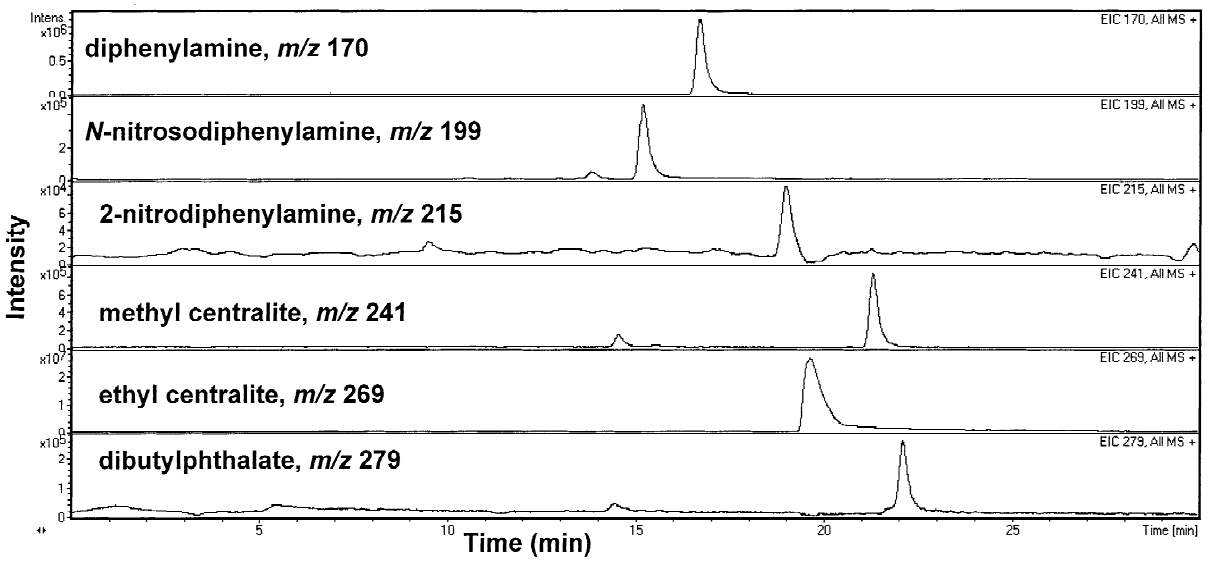

Fig. 3. Extracted ion chromatograms of Red Dot 900 smokeless powder showing 2-nitrodiphenylamine, *m*/*z* 215, without the 4-nitrodiphenylamine isomer. Conditions as listed in Table 1 and Fig. 1.

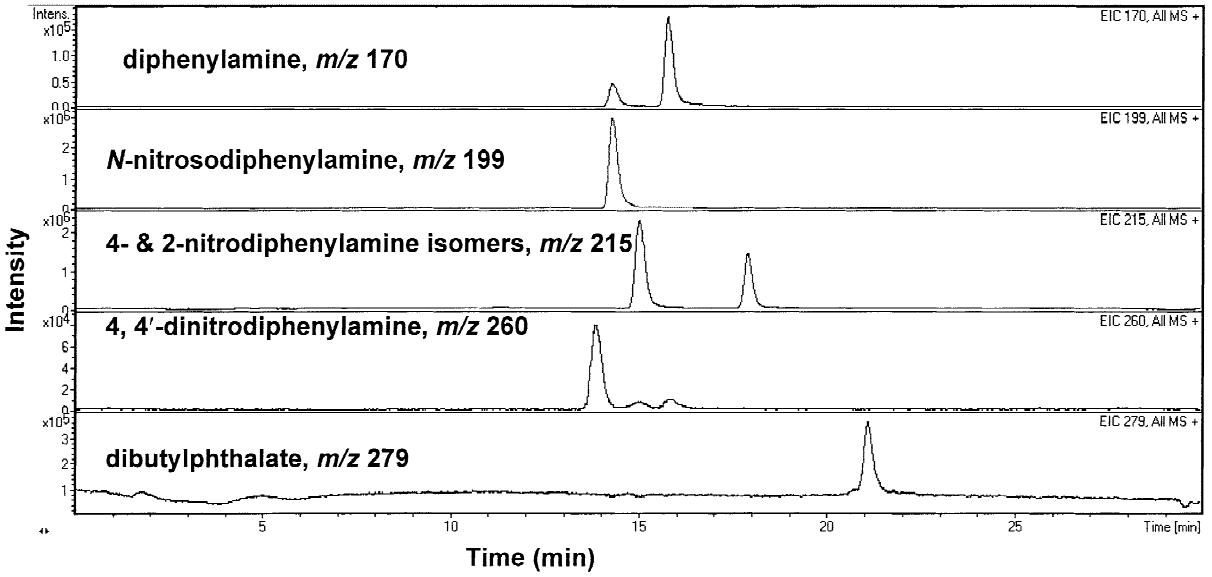

Fig. 4. Extracted ion chromatograms of IMR 4895 smokeless powder, in which diphenylamine, *N*-nitrosodiphenylamine, 4- and 2-nitrodiphenylamine isomers, 4,4′-dinitrodiphenylamine and dibutylphthalate were detected. Conditions as listed in Table 1 and Fig. 1.

4-4′-DNDPA and DBP were detected. The mass spectrum of 4-4′-DNDPA shown in Fig. 5 illustrates the detection of the protonated molecule of 4-4′-DNDPA, m/z 260, and the minor presence of its sodium adduct, m/z 282. The detection of the 2NDPA isomer and 4-4′-DNDPA demonstrates the effectiveness of the LC-ESIMS method for the detection of minor compositional changes in smokeless powders.

The results from this study were used to identify distinct compositions among smokeless powders. The identification of the components with the gradient reversed-phase LC-ESIMS method produces a profile of the different additives in smokeless powders. This profile has been used for the comparison of different commercially available powders. Previous studies on smokeless powder additives from GSR with ESIMS/MS, quantified MC as well as DPA, *N*sDPA and 4NDPA to demonstrate ESIMS/MS detection, but only determined these additives in a limited number of smokeless powders [11,12]. In this study, the gradient reversed-phase LC-ESIMS method was employed to characterize smokeless powders by simultaneously quantifying several organic additives including diphenylamines, centralites, and phthalates.

4. Conclusions

The goal in the forensic analysis of smokeless powders is to characterize and identify individual constituents. The current method was developed to establish an LC-ESIMS method for the simultaneous determination of the common organic additives in smokeless powders. The ESIMS was optimized for the detection of protonated molecules in the full scan positive ion mode. The separation of compounds with varying polarity was accomplished by using the octyl column with the methanol gradient. For example, *N*-nitroso-, 4-nitroso-, 4-nitro- and 2-nitro-derivatives of diphenylamine, which are reaction products of nitrous and nitric acid with DPA, were separated and quantified. The gradient reversed-

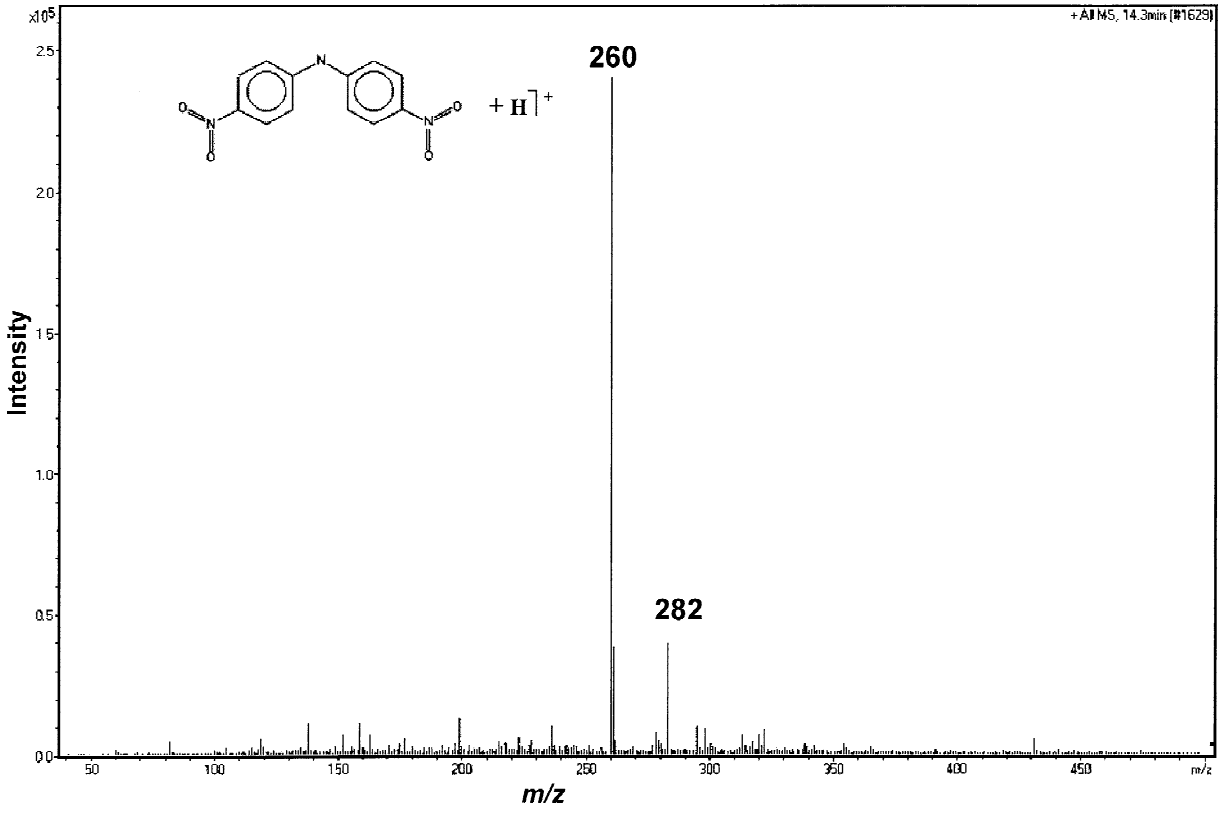

Fig. 5. Mass spectrum of 4-4′-dinitrodiphenylamine, collected during chromatographic run of IMR 4895. Conditions as listed in Table 1 and Fig. 1.

phase LC-ESIMS method was used to differentiate several unburned powders by their additive profile. Because quantitative validation and test sample results were obtained for the selected components, the method developed in this study should prove useful in the analysis of compositional variation and smokeless powder degradation.

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