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DOI
10.1016/j.chroma.2022.463072

Publication date
2022

Document Version
Final published version

Published in
Journal of Chromatography A

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Journal of Chromatography A 1672 (2022) 463072

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Characterization and comparison of smokeless powders by on-line two-dimensional liquid chromatography

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ARTICLE INFO

Article history:
Received 20 January 2022
Revised 13 April 2022
Accepted 14 April 2022
Available online 16 April 2022

Keywords:
Nitrocellulose
Smokeless powder
Forensic explosives analysis
Two-dimensional liquid chromatography

ABSTRACT

Smokeless powders (SPs) are one of the most commonly used propellants for ammunition but can also be abused as energetic material in improvised explosive devices (IEDs) such as pipe bombs. After a shooting or explosion, unburnt or partially burnt particulates may be observed which can be used for forensic investigation. SPs comprise mainly nitrocellulose (NC) and additives. Therefore, the characterization of both NC and the additives is of significant forensic importance. Typically, the identification, classification, and chemical profiling of smokeless powders are based exclusively on the analysis of the additives. In this study, information regarding the NC base component was combined with the chemical analysis of the additives using two-dimensional liquid chromatography (2D-LC). The system combines size-exclusion chromatography (SEC) and reversed-phase liquid chromatography (RPLC) in an on-line heart-cut 2D-LC configuration. In the first dimension, the NC is characterized by its molecular-weight distribution (MWD) while being separated from the additives. The additives are then transferred to the second-dimension separation using a novel analyte-transfer system. In the second dimension, the additives are separated to obtain a detailed profile of the low-molecular-mass compounds in the SP. With this approach, the MWD of the NC and the composition of the additives in SP have been obtained within an hour. A discrimination power of 90.53% was obtained when studying exclusively the NC MWD, and 99.47% for the additive profile. This novel combination enables detailed forensic comparison of intact SPs. Additionally, no extensive sample preparation is required, making the developed method less labor intensive.

1. Introduction

Smokeless powders (SPs) are low explosives typically used as a propellant for ammunition but are also encountered in improvised explosive devices (IEDs) (*e.g.* pipe bombs and pressure cooker IEDs) [1]. SP-based IEDs are of concern among law enforcement agencies especially in the USA where SPs are readily available [2]. In some cases, IEDs can be defused in time, enabling detailed forensic investigation of the individual parts, including the main charge. However, after successful activation of the device, SP residues can also be recovered for analysis [3,4]. The identification of SP residues is of importance in post-explosion investigations to reconstruct the device used [5,6]. In addition, when forensic experts encounter a significant amount of intact SP in a pre-explosion investigation, establishing a chemical profile of the propellant could enable a forensic comparison with energetic material found in a suspect's home or originating from another case. Moreover, improved characterization of SPs may be used for quality-control (QC) purposes during the manufacturing process.

There are three categories of SPs: single-base, double-base, and triple-base. Single-base SPs contain almost exclusively nitrocellulose (NC) which is the main propellant in all three types of powders. Double-base SPs also contain nitroglycerin (NG) and in triple-

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https://doi.org/10.1016/j.chroma.2022.463072

base SPs, the propellant mix consists of NC, NG, and nitroguanidine (*e.g.* to slow down deterioration). Single- and double-base SPs are easily accessible to consumers in certain geographical areas (notably the USA and other countries where few restrictions exist for the possession and use of firearms) whereas triple-base powders are primarily used in military-grade weaponry [7,8]. Nitrocellulose is a structurally complex high-molar-mass polymer that is produced by the nitration of plant-based cellulose using concentrated nitric and sulfuric acid [9]. Additional components include a variety of additives such as stabilizers, deterrents, plasticizers, and flash inhibitors [10–13]. These additives are used to control properties such as the burn rate and shelf life of the product [14].

The identification of common additives in SPs has been widely researched, especially in unburned propellant powders. Already in the early 2000s, several forensic science teams investigated the analysis of SP additives [15,16]. Wu et al. [17] reported the use of tandem mass spectrometry (MS/MS) for detecting methyl centralite (MC) and later demonstrated the analysis of diphenylamine (DPA) stabilizer and its nitrated derivates in SPs using MS/MS [18]. In 2009, extraction of additives using methanol followed by nano electrospray ionization MS was used to characterize SPs based on the additive composition [19]. Wissinger and McCord [20] demonstrated the use of reversed-phase liquid chromatography (RPLC) coupled to ultraviolet (UV) detection for separating several common SP additives in a single analysis. Later, Laza et al. [12] used LC coupled to MS/MS for quantitative analysis of ethyl centralite (EC), DPA, MC, N-nitrosodiphenylamine (N-NsDPA), 2-nitrodiphenylamine (2- NDPA), and 4-nitrodiphenylamine (4-NDPA) in gunshot residues retrieved from the hand of a shooter. A solid-phase extraction (SPE) sample preparation step was required for the purification and concentration of the analytes. In 2013, Thomas et al. [11] introduced an LC-MS/MS method, that was capable of analyzing a broad range of SP additives. Subsequently, Benito et al. [13] reported an LC method coupled to quadrupole time-of-flight (qTOF) MS for organic gunshot residues. In addition to LC-based methods, gas chromatography has also been used by several researchers to assess additive composition [5–7]. All of the methods described above require sample preparation to extract the additives and disregard the nitrocellulose component of the SPs. Rather than exploring the NC as a source of forensic information, nitrocellulose is seen as an obstructing matrix that needs to be removed to prevent interference.

Although the additives in SPs yield useful chemical fingerprints that can be used for forensic identification and comparison purposes, additive compositions are governed by product specifications and are strictly controlled resulting in limited variation across batches [21]. Therefore, including the chemical characterization of NC itself, might significantly increase the options for forensic comparison and attribution given the fact that it is produced from cellulose obtained from natural sources. Size-exclusion chromatography (SEC) is a useful and robust technique to study the molecular-weight distribution (MWD) of nitrocellulose [9,22]. To assess the MWD, calibration with polymer standards is desired which is complicated by the fact that well-defined NC polymer standards are not available. Still, SEC has been applied successfully for the analysis of nitrocellulose before by using polystyrene standards as reviewed by de la Ossa et al. [9]. However, to our knowledge, the NC MWD has not been used for forensic comparison before. This work investigates whether the combination of two orthogonal chemical features of SPs (NC MWD and additive profile) could significantly improve the options for forensic comparison and attribution.

To obtain information about these two orthogonal sample features, two-dimensional (2D) LC could be explored. The combination of organic SEC and RPLC, with SEC as a first-dimension (^{1}D) separation, in a 2D-LC system has a high likelihood of breakthrough as detailed by Pirok et al. [23]. Moreover, the additives may be present at low concentration which introduces the additional problem of transferring a large volume of ^{1}D effluent while minimizing the elution peak volume in the second-dimension (^{2}D) [24]. Active modulation may be used to overcome ^{2}D breakthrough and analyte focusing. Active modulation approaches include active-solvent modulation (ASM) [25], stationary-phase-assisted modulation (SPAM) [26], and thermal modulation [27]. Polymer separations have been performed previously by comprehensive 2D-LC (LC × LC) with organic SEC as ^{1}D using ASM [28,29]. While these approaches demonstrate the power of ASM to prevent breakthrough when using strong organic ^{1}D eluent, the use of LC × LC features low-volume ^{1}D effluent fractions. To the best of our knowledge, SEC and RPLC have not previously been combined in a large-volume heart-cut format that overcomes both breakthrough and sensitivity issues.

In this work, an on-line heart-cut 2D-LC method is proposed using SEC as a ^{1}D separation method and RPLC as a ^{2}D separation mechanism for a comprehensive characterization of SP samples that does not require extensive sample preparation. We will first address the use of the individual one-dimensional (1D) separation methods. Next, the development of a novel analyte-transfer system, to enable on-line coupling of organic SEC and RPLC, will be described. Finally, the performance of the developed method will be demonstrated using two SPs measured ten-fold. Finally, a total of twenty SPs were compared based on NC MWD and additive formulation. By characterizing both the NC MWD and additive profile, the evidential value of matching profiles was significantly increased.

2. Materials and methods

2.1. Chemicals

All water referred to in this article was deionized (Arium 611UV; Satorius, Germany; resistivity 18.2 MΩ. cm). Unstabilized tetrahydrofuran (THF, HPLC grade), methanol (MeOH, ULC/MS – CC/SC grade), and acetonitrile (ACN, LC-MS grade) were obtained from Biosolve (Valkenswaard, The Netherlands). Dichloromethane (DCM) was obtained from VWR chemicals (Fontenay-sous-Bois, France). Polystyrene standards (PS) for SEC calibration were obtained from Polymer Laboratories (now Agilent Technologies, Church Stretton, Shropshire, UK). All additive reference standards: diphenylamine (DPA), N-nitrosodiphenylamine (N-NsDPA), 2-nitrodiphenylamine (2- NDPA), 4-nitrodiphenylamine (4-NDPA), methyl centralite (MC), ethyl centralite (EC), dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), nitroglycerin (NG), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 3,4-dinitrotoluene (3,4-DNT) were obtained from AccuStandard (New Haven, Connecticut, USA). Formic acid (FA, ≥98%) and 2-naphthol were obtained from Merck (Darmstadt, Germany).

Twenty SPs were obtained from the US National Center for Forensic Science (NCFS) Smokeless Powders Database Reference Collection [21].

2.2. Instrumentation

All experiments in this study were carried out using an Agilent 1290 series Infinity 2D-LC system (Agilent, Waldbronn, Germany). The system comprised of two binary Infinity I pumps (G4220A), one high speed Infinity II pump (G7120A), one 1200 isocratic pump (G1310A) with an 1100 degasser (G1379A), an autosampler (G4226A), a thermostatted column compartment (G1316C) and a valve drive (G1170A), both equipped with an 8-port 2-position 2D-LC valve (G4236A) of which one was equipped with two 40 µL

stainless steel sample loops for 2D-LC (P/N: 5067–5425) and two diode-array detectors (G4212A) (DAD) with Agilent Max-Light cartridge cells (G4212–60,008, 10 mm, V_{det} = 1.0 µL). The autosampler injector needle was set to draw and eject at 100 µL min^{-1} with an equilibration time of two seconds for all experiments. All tubing and connections were made from stainless steel. The system was controlled using Agilent OpenLAB CDS Chemstation Edition (Version 3.2 (Build 3.2.0.620)) software. For 2D-LC experiments, a Shimadzu stainless-steel SUS variable volume mixer (P/N: 228–45,093–91) (Shimadzu, Canby, USA) and a Waters 50 µL zirconia mixer (P/N: 700,002,911) were added to the system.

The ^{1}D column was an Agilent PLgel MiniMix-C (250 × 4.6 mm i.d., 5 µm, P/N: PL1510–5500). The ^{2}D column was a Waters Acquity UPLC BEH C18 (100 × 2.1 mm i.d., 1.7 µm, SKU: 186,002,352). As a trap column, an Agilent ZORBAX RRHD Eclipse Plus C18 (50 × 2.1 mm i.d., 1.8 µm, P/N: 959,757–902) was used. In one experiment, the Agilent column was exchanged for a Phenomenex SecurityGuard ULTRA Holder (P/N: AJ0–9000) equipped with a UHPLC C18 cartridge (2 × 3.0 mm i.d., P/N: AJ0–8775).

2.3. Procedures

2.3.1. Sample preparation

For 1D-SEC experiments, the SP samples were dissolved in unstabilized THF at a concentration of 1 mg mL^{-1} and left overnight to dissolve.

For 1D-RPLC experiments, extraction was performed using DCM as described by Wissinger and McCord [20]. A total of 20 mg SP was suspended in 1 mL DCM and left overnight to extract the additives. A volume of 400 µL of the resulting solution was transferred into a vial and dried using a gentle stream of N_2, the remains were redissolved in 800 µL of H_2O:ACN (60:40 v/v).

For the 2D-LC experiments, the SP samples were dissolved at a concentration of 1 mg mL^{-1} in unstabilized THF containing 0.005 mg mL^{-1} 2-naphthol (ISTD) and left overnight to dissolve.

2.3.2. One-dimensional separations

For the 1D-SEC-UV experiments, the isocratic pump was connected to the autosampler, which was then connected to the PLgel MiniMix-C column and linked to the DAD. Unstabilized THF containing 5 mM FA was used as mobile phase, the flow rate was set at 0.3 mL min^{-1}. At this flow rate, the recorded pressure was typically around 23 bar. The total analysis time was 12 min. The injection volume was set at 20 µL. The DAD monitored the chromatogram at 210 nm as detection wavelength (bandwidth 4 nm) at a sampling rate of 2.5 Hz. However, the full UV-spectra from 210 to 400 nm were also recorded and stored.

For 1D-RPLC-UV experiments, one of the binary pumps was attached to the autosampler to which the Waters Acquity UPLC BEH C18 column was connected and subsequently coupled to the DAD. The solvent compositions and gradient program were based on the work of Thomas et al. [11]. Mobile phase A was a mixture of H_2O:ACN (90:10 v/v) containing 5 mM FA. Mobile phase B was a mixture of ACN:MeOH in a (95:5 v/v) containing 5 mM FA. The flow rate was set at 0.5 mL min^{-1} and the flow was directed through the 6 µL heater in the column compartment. Both the heater and the column were kept at 50 °C to lower the operating pressures. The exact gradient program is presented in Table S-1 of Supplementary material. The total analysis time was 10 min. An injection volume of 20 µL was employed. The DAD recorded the chromatogram at 210 nm as detection wavelength (bandwidth 4 nm) at a sampling rate of 40 Hz, while full UV-spectra from 210 to 400 nm were also stored for differentiating the chemical identity of the investigated additives.

2.3.3. On-line heart-cut SEC-RPLC

A schematic overview of the instrumental setup used for the heart-cut SEC-RPLC experiments is depicted in Fig. 1. The ^{1}D detector (DAD_1) and the ^{2}D detector (DAD_2) were operated at 2.5 and 40 Hz respectively. Chromatograms were recorded at 210 nm and the full UV spectra from 210 to 400 nm were stored. Both of the 2D-LC valves were used in concurrent mode, meaning that the 40 µL loops for the left valve ($Valve_1$) and the trap column ($RPLC_1$) in the right valve ($Valve_2$) were both filled and emptied in the same flow direction. To $Valve_2$, a low-volume piece of tubing (∼ 1.1 µL) was connected to effectively turn the 8-port valve into a 6-port valve. This modification was necessary because a conventional 6-port valve able to operate above 800 bar was not available. The first mixer ($Mixer_1$) had a mixing volume of 0.5 mL and the second mixer ($Mixer_2$) had a volume of 50 µL.

A detailed overview of the time-programming of each module in the system and subdivision into the four phases of the method is presented in Fig. 2 and Tables S-2, S-3, and S-4. During the SEC phase, $Pump_1$ operated at a flow rate of 0.3 mL min^{-1} THF with 5 mM FA for 9.5 min. $Pump_2$ and $Pump_4$ operated at 100% B for 6 min at flow rates of 0.2 and 0.5 mL min^{-1}, respectively to regenerate $RPLC_1$ and $RPLC_2$. Then, both pumps changed to 100% A where $Pump_2$ changes to a flow rate of 0.8 mL min^{-1} to be ready for the next phase where this flow was used to dilute the SEC effluent. $Pump_4$ changes to 0.03 mL min^{-1}.

During the trapping phase, $Pump_1$ lowered its flow rate to 0.04 mL min^{-1} to enable sufficient dilution of the SEC effluent for analyte trapping on the RPLC column. $Valve_1$ switched 30 times, every 30 s, starting at 10 min to transfer SEC effluent fractions of 20 µL to the dilution flow and $Mixer_1$ for dilution at a 1:20 ratio.

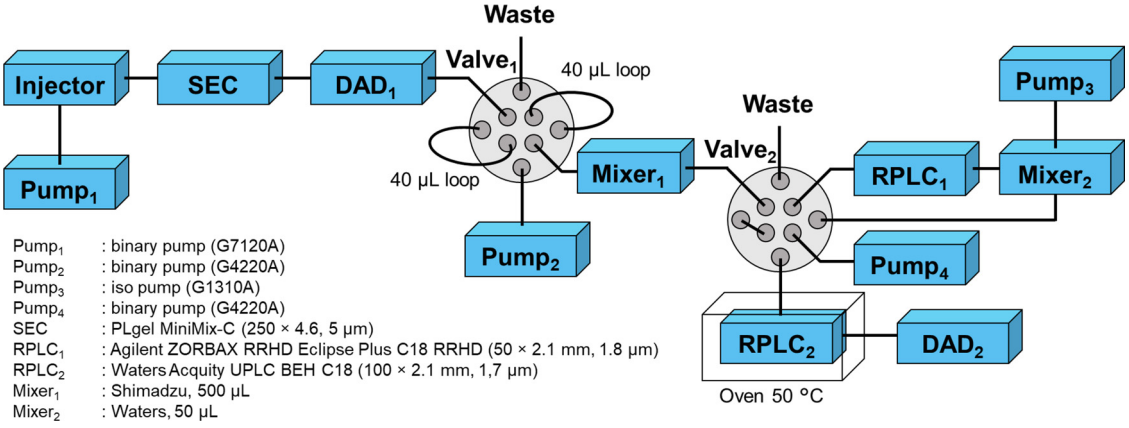

Fig. 1. Schematic overview of the heart-cut SEC-RPLC setup used for characterizing smokeless powders.

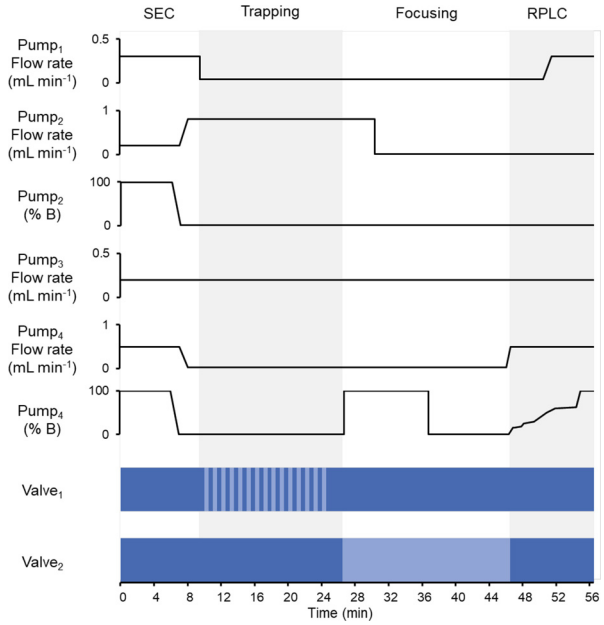

Fig. 2. An overview of the time programming of each module in the heart-cut 2D-LC system. For the valves, dark blue indicates position 1 and light blue indicates position 2. The method is subdivided into four phases: SEC, Trapping, Focusing, and RPLC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

During the focusing phase, $Pump_2$ reduced its flow rate to 0.01 mL min^{-1} to preserve solvent. $Valve_2$ was switched to direct the flow from $Pump_4$ to $RPLC_1$. $Pump_4$ changed to 100% B for 10 min to elute the trapped additives from $RPLC_1$. Afterward, $Pump_4$ changed back to 100% A and at 45 min it increased its flow rate to 0.5 mL min^{-1} to prepare for the next phase. $Pump_3$ operated at a flow rate of 0.2 mL min^{-1} at 100% H_2O to dilute, at a 3:20 ratio using $Mixer_2$, and subsequently focus the $RPLC_1$ effluent onto $RPLC_2$. The optimization of these elution conditions is explained in Section 3.2.

During the RPLC phase, $Valve_2$ switched back to position 1 such that the flow of $Pump_4$ is directed to $RPLC_2$. Simultaneously, the ^{2}D gradient program was started which was similar to that in Fig. 3B but remained at 100% B at the end to prepare for the next measurement. Additionally, $Pump_1$ increased its flow rate to 0.3 mL min^{-1} at 50 min to equilibrate for the next measurement.

2.3.4. Data handling

The SEC chromatograms were converted to PS-calibrated MWD by converting the time-axes to a Log(MW) axes to display the MWDs. For all the 2D RPLC chromatograms, a blank measurement was subtracted due to the presence of some system impurities of unknown origin. This subtraction was performed using MATLAB R2021a by aligning the most-abundant peak in the blank (see Fig. S-1) with the same peak in the sample before subtracting the blank from the chromatogram. Afterward, the additive peaks were identified by using the Findpeaks function (part of the Signal Processing toolbox, R2021a) to obtain the local peak maxima, being the 2D retention times, and peak width at half height (2.35 σ). To integrate the peaks that correspond with the known retention times of the additives, a window was integrated that was equal to 4.7 σ using trapezoidal numerical integration (trapz function, R2021a). To calculate the wt% of each additive, the areas of the pure additive standards were used as one-point calibration followed by correction for the peak area of the internal standard (ISTD) 2-naphthol which was added to every sample at a known concentration.

3. Results and discussion

3.1. One-dimensional separations

SEC separations are commonly carried out on columns with a relatively large volume (in the order of several mL) as the separation power is proportional to the total pore volume [30]. However, for the purpose of this study, the aim was to keep the elution volumes relatively small due to the low flow rates that are typically used in the ^{1}D separation of a 2D-LC system. Using the MiniMix-C column, the elution volume of both the nitrocellulose and the additives was kept relatively low as the total analysis time at a flow rate of 0.3 mL min^{-1} was 12 min, meaning that the total elution volume was roughly 3.6 mL. Using 14 PS standards measured in triplicate, a calibration curve was constructed (see Figs. S-2A and S-2B) to calculate the PS-calibrated molecular-weight distribution (PS-c MWD) of the NC (see Fig. S-2C). It should be noted that, in the absence of suitable low-polydispersity NC standards, an accurate determination of the MWD was challenging. However, the purpose of this method was to compare MWD of NC in SP samples with high precision, which is possible with PS calibration. Nevertheless, the degree of nitration of NC may affect its extinction coefficient and with that the shape of the weight distribution. This was investigated by inspecting the full, normalized DAD spectra which illustrated that the extinction coefficient was consistent over the entire MWD of an SP sample (see Fig. S-3). This indicated that the shape of MWD is not influenced by the nitration degree. To emphasize that the MWD of NC as established using this method will systemically deviate from the true distribution, the term PS-c MWD will be used. Because the additives are all low-MW species, they will elute from the column after the NC. As observed from a 1D-SEC measurement of an SP sample (see Fig. 3A), the additives appear to elute between 9.5 and 11.5 min, corresponding to a volume fraction of 600 µL.

Reversed-phase liquid chromatography (RPLC) was used for the separation of the additives present in the smokeless powder samples. To assess the separation capabilities of the method, 1D-RPLC measurements were conducted using an off-line extraction method of the additives. Two additive fractions of smokeless powders were

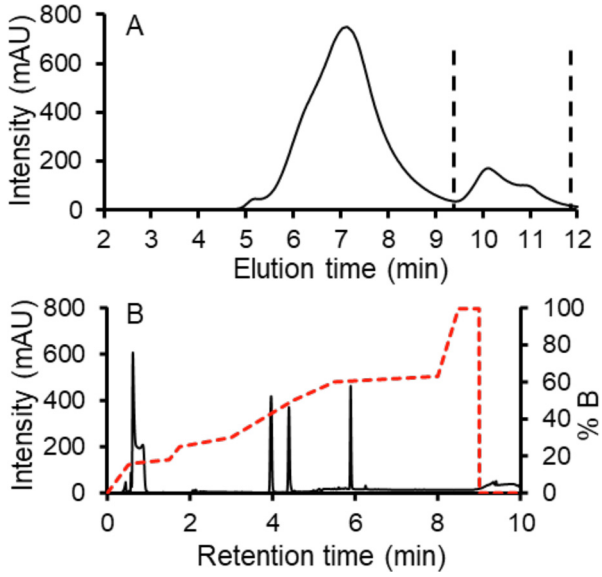

Fig. 3. SEC chromatogram of an SP sample 355 (A) and the 20 µL reconstituted additive fraction in RPLC (B). The red dashed line in B represents the solvent-B% as a function of time to illustrate the gradient program used for 1D-RPLC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subjected to analysis (see Fig. S-4). From those measurements, all compounds could be recognized by a combination of their RPLC retention time and corresponding UV spectrum as was confirmed by measuring the individual additive standards (see Figs. S-5 and S-6). The retention times of 2-NT and 3,4-DNT were nearly identical, their UV spectra are however slightly different and they may be deconvoluted using the DAD spectra.

To investigate the feasibility of hyphenating the aforementioned SEC and RPLC methods for smokeless powder characterization, the first experiments were conducted in an off-line manner. In this experiment, the additive fraction was manually collected from 9.5 until 11.5 min at the outlet of the DAD used for a 1D-SEC analysis (Fig. 3A) of a smokeless powder sample. Subsequently, this fraction of additives in roughly 600 µL THF was evaporated to dryness under a gentle stream of N_2 gas and the remains were redissolved in 50 µL of H_2O:ACN (60:40 v/v). Finally, 20 µL of this solution was subjected to the 1D-RPLC method described above (Fig. 3B). For this off-line 2D-LC experiment, the same sample was used that featured in Fig. S-5A. Comparison of the two RPLC separations demonstrates similar additive composition. It should be noted that in this off-line experiments some unknown contamination was observed at 1 min. This was assumed to an artifact of the manual off-line methodology and therefore ignored. This suggests that the additives did elute in the volume that was manually collected during the 1D-SEC measurement and that they can be concentrated and subjected to a ^{2}D RPLC separation without significant analyte loss. Moreover, the off-line 2D-LC experiment demonstrated that it would be possible to simultaneously characterize the MWD of the NC in SPs as well as the additive composition, two orthogonal sample features. Preferably, this process should be automated in an on-line manner using a novel modulation interface thereby minimizing manual sample preparation and risk of laboratory errors.

3.2. Development of the modulation interface

The SEC and RPLC separations were coupled using the instrumental setup presented in Fig. 1. The coupling of the 1D methods introduced several challenges. As explained above, the additives elute from SEC in a volume of roughly 600 µL. To ensure that all additives are subjected to the ^{2}D RPLC separation, this entire fraction of additives in 600 µL of THF must be sampled and transferred to the ^{2}D column while maintaining separation power in the second dimension. THF is a notoriously strong solvent in the context of RPLC retention mechanisms. Therefore, additives dissolved in THF may exhibit breakthrough when the two separation dimensions would simply be hyphenated without a dedicated analyte-transfer system.

To overcome this problem, the SEC effluent was sampled in fractions of 20 µL at a time and diluted with a high flow rate of water ($Pump_2$) at 0.8 mL min^{-1} such that the analytes could be retained on the trapping column ($RPLC_1$). To enable such fractioning and trapping, the flow rate of the ^{1}D separation had to be lowered to 40 µL min^{-1}. This way, the 2D-LC valve could switch every 30 s to subject a 20 µL fraction of additives in THF to the mixer and subsequently the trapping column. Another reason to use a 2D-LC valve for this purpose was to ensure that the high pressure (roughly 750 bar) created by the high dilution flow rate towards the trapping column does not damage the DAD or the ^{1}D SEC column which have pressure limits of only 70 and 150 bar, respectively. The analytes are initially contained on a trapping column instead of directly on the ^{2}D column due to peak broadening that occurs during trapping as a result of repeated THF injections.

To investigate on-column trapping, the SEC dimension was omitted and a six-component standard mixture of DMP, NG, MC, DEP, EC, and DBP was prepared for testing purposes at concentrations of 0.05 mg mL^{-1} each in THF. Where DMP is the least-retained and DBP is the most-retained compound on RPLC out of all additives investigated in this study. In this setup, a binary pump was connected to the autosampler which was coupled to a 2D-LC valve to direct the flow either through the 0.5 mL mixer and subsequently the ^{2}D column, or to direct the flow through the column, bypassing the mixer and reducing dwell volume for gradient elution. Finally, the ^{2}D column was connected to a DAD (see Fig. S-7). To simulate 20 µL fractions being sampled, 20 µL of the standard mixture was injected using a flow of 100% H_2O through the mixer and subsequently onto the column. The valve was then switched and the gradient program was started as described in the previous section. To simulate multiple fractions being trapped, multiple injections of 20 µL were performed before switching the valve and starting the gradient elution (see Fig. 4). Due to significant peak broadening during trapping, it was necessary to perform trapping and focusing as two separate stages in the overall method.

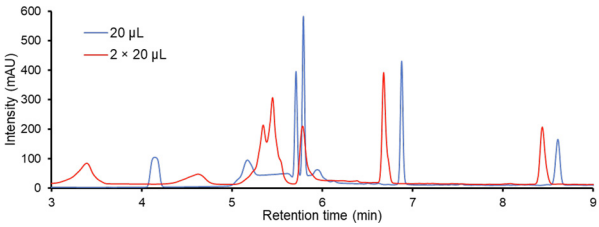

Fig. 4. ^{2}D RPLC separations using on-column trapping of a mixture of six standards dissolved in THF by performing a 20 µL injection (blue) and performing two subsequent injections of 20 µL each (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

It was also investigated whether the trap column (50 × 2.1 mm i.d.) could be replaced by a small-volume trap (2 × 3.0 mm i.d.). However, contrary to the larger-volume trap column, the additives were found to be insufficiently retained on the small-volume trap as these compounds were no longer observed in the ^{2}D RPLC separation (see Fig. S-8). It should be noted that these traps were from different manufacturers and therefore the selectivity may have also varied. However, for the purpose of this two-stage modulation interface, it is not necessary that the selectivity of the stationary phase is identical to the column used in the second dimension as the trap column is not involved in the actual separation of the analytes. Therefore, the 50 × 2.1 mm i.d. C18 column was considered optimal to trap the additives from a total volume fraction of 600 µL THF using the proposed setup.

To optimize elution from the trap column, the following setup was used, similar to Fig. 1: $Pump_2$ was connected to the injector and subsequently to $Mixer_1$ thereby omitting SEC and $Valve_1$ (see Fig. S-9). In both of the above-mentioned systems, the same gradient as in Fig. 3B was used for elution of the additives while the DAD was operated using the same settings as for the 1D RPLC experiments. Step 1: a 20 µL injection of the six-component standard mixture was performed and directed through the mixer and subsequently to $RPLC_1$. Step 2: the valve switched, $Pump_4$ pumped 100% mobile phase B at 30 µL min^{-1} for 10 min to $RPLC_1$. The analytes were diluted and mixed using $Pump_3$ (100% water at 0.2 mL min^{-1}) and $Mixer_2$ and were finally focused on $RPLC_2$. Step 3: the valve switched, $Pump_4$ started with the ^{2}D gradient.

The effect of elution flow rate and duration was studied and the results are displayed in Fig. 5. As can be seen from this Figure, the elution volumes of mobile phase B were varied at 150, 225, and 300 µL. This was achieved by either using a lower flow rate or altering the elution time. The results demonstrated inadequate transfer of highly retained analytes, like EC and DBP, when using lower elution volumes. Moreover, the retention times of the poorly retained analytes, like DMP and NG, were further reduced. Nevertheless, this did not seem to alter the sensitivity of these analytes.

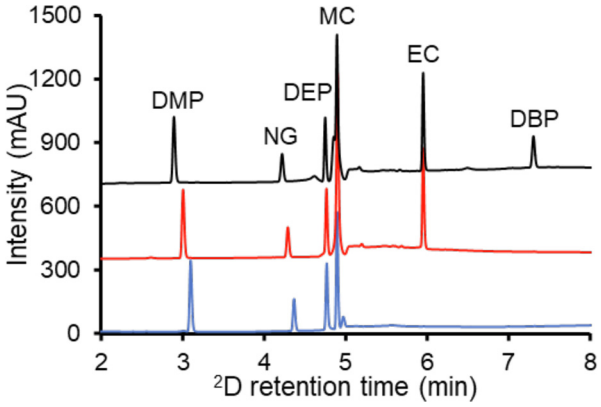

Fig. 5. ^{2}D RPLC separation of a six-component standard mixture using different trap-elution conditions, 20 µL min^{-1} mobile phase B was used for 7.5 min (blue), 30 µL min^{-1} for 7.5 min (red), and 30 µL min^{-1} for 10 min (black). The red and black chromatograms are offset on the y-axis for illustration purposes by 350 and 700 mAU, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

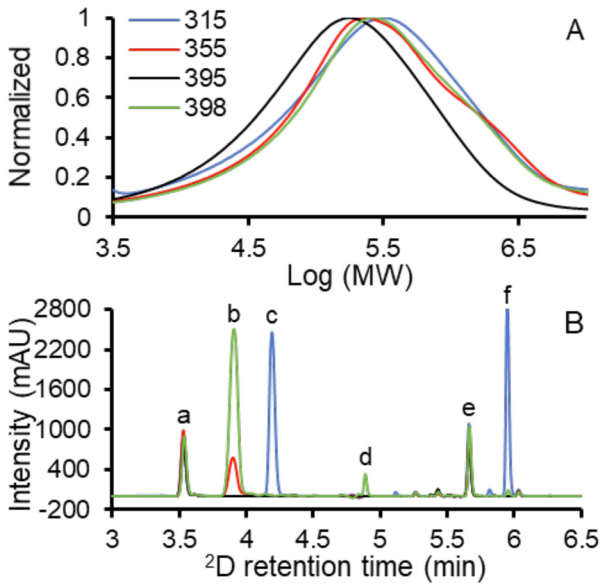

Fig. 6. PS-calibrated MWD obtained from the 1D SEC (A) and the 2D chromatogram from 3 to 6.5 min where the additives elute (B). Here, the labelled peaks are identified as: 2-naphthol (a), 2,4-DNT (b), NG (c), MC (d), DPA (e), EC (f). The color legend for both subfigures is included in graph A and indicates the NCFS database code for the commercial SPs.

Therefore, it was concluded that the above-mentioned conditions were optimal, using an elution volume of 300 µL. However, analyte transfer now significantly adds to the total analysis time with both the ^{1}D and ^{2}D separations taking roughly 10 min individually whereas the final analyte-transfer method encompasses 15 min of ^{1}D effluent fractionation and trapping followed by 18 min of trap elution and analyte focusing on the ^{2}D column. This highly complex analyte-transfer system was far from ideal but proved to be necessary as demonstrated above where simpler instrumental setups did not suffice for this application. Nevertheless, errors in calculated additive contents might be reduced by using an ISTD in every sample in addition to calibration using the full 2D-LC setup.

3.3. On-line heart-cut SEC-RPLC

A number of smokeless powder sample solutions all at concentrations of 1 mg mL^{-1} with 2-naphthol as ISTD at a concentration of 0.005 mg mL^{-1} were individually injected and analyzed using the developed heart-cut 2D-LC separation system. Fig. 6 shows overlays of the obtained PS-c MWD from ^{1}D SEC (Fig. 6A), and the ^{2}D RPLC chromatograms (Fig. 6B) of four different smokeless powders samples from the NCFS collection. Variations in the PS-c MWD were observed for these four samples and sample 395 revealed significant differences compared to the others. For samples 355 and 398, however, the difference in PS-c MWD was minimal.

Nevertheless, when studying the ^{2}D separation as well, significant differences in the additive composition were apparent between all samples. The ISTD (2-naphthol, at 3.52 min) was visible in all samples. Most notably, sample 315 stands out the most in both the ^{1}D and ^{2}D separations. A large amount of nitroglycerin was present in this sample, revealing its double-base nature whereas the other three samples were classified as single-base powders due to the absence of this organic nitro-ester. Additionally, the dominant presence of EC was characteristic for this sample. Sample 395 also exclusively contained MC in this small sample set, DPA however appeared to be present in all four samples at a similar concentration. DPA as well as EC and MC are used as stabilizers in smokeless powders [13]. These results suggest that DPA might be less suitable for SP differentiation. Sample 355 and 398 both contained 2,4-DNT, which is used as a flash inhibitor, but at significantly different levels. Aside from the most obvious peaks in the ^{2}D chromatogram, the presence of distinctive constituents at trace levels was also observed including, 4-NDPA and N-NsDPA, which are known degradation products that can be present in the SP in small amounts. These degradation products can be very valuable features to distinguish products of the same brand and manufacturer that have a different shelf life and/or storage conditions. Overall, these four samples suggest the potential power of the developed 2D-LC separation system to assess both the MWD of the NC as well as the composition of the additives simultaneously. Moreover, no extensive sample preparation was required compared to the 1D-RPLC analysis of the additives. Simply dissolving the whole sample in THF with 2-naphthol as ISTD was sufficient for analysis by the SEC-RPLC system.

Moreover, to assess the robustness of the method, samples 315 and 395 have been measured tenfold over the course of a week. Using this data, the average SEC chromatogram was calculated with its corresponding standard deviation (SD) at every data point, the results are displayed in Fig. 7A. The corresponding normalized PS-c MWDs are depicted in Fig. 7B. The results demonstrate that these samples can confidently be differentiated by their MWD.

The ^{2}D RPLC chromatograms of the ten-fold measurements were used to calculate the additive contents in weight percentage (wt%) using one-point external calibration with and without ISTD correction (see Table S-5). The average wt%, the SD of the wt% and the relative SD (RSD) were calculated for all identified additives in both samples. The results demonstrated that the errors can be greatly reduced by using a one-point calibration corrected for the ISTD peak area. However, when the analyte was present at low levels (below 0.3 wt%), the RSD becomes substantial although the absolute error in wt% remains acceptable. Therefore, quantification of low-abundant additives was challenging and unreliable but the presence of these additives was confirmed using the corresponding UV spectra. The presence of such traces could therefore still be used qualitatively to compare SP samples.

3.4. Comparison of smokeless powders from different manufacturers

Twenty SP samples obtained from the NCFS SP database were measured in triplicate using the heart-cut SEC-RPLC system. The SEC and RPLC data were compared separately and the SEC comparison is presented in Table 1. For the comparison of the NC PS-c MWDs, the SEC chromatograms were intensity normalized to avoid

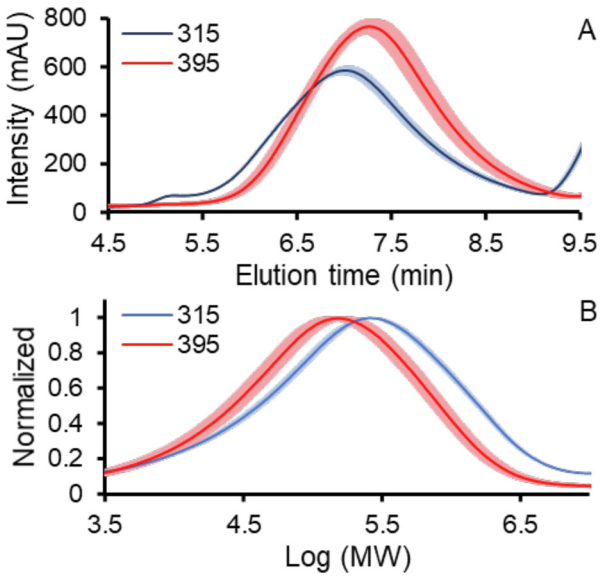

Fig. 7. Average ^{1}D SEC chromatograms of samples 315 (blue) and 395 (red) calculated from ten-fold measurements over the course of a week (A) and the corresponding PS-calibrated MWD on a normalized intensity scale (B). The light-shaded area represents one SD over the entire chromatograms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Pair-wise comparison of the sum of all residuals from normalized 1D SEC MWDs of twenty SPs, identified by their sample-reference number (SRN). A red highlight indicates a sum of residuals from the PS-c MWD comparison below 2 SD ('indistinguishable'), a yellow color indicates a residual between 2 and 5 SD ('most likely different') and green shaded cell represent residuals exceeding 5 SD ('clearly different').

SRN	315	355	398	395	304	303	335	349	310	324	334	308	317	312	330	311	325	326	309	333
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bias resulting from total additive content. The MWDs were compared pair-wise where one MWD was subtracted from the other MWD to obtain the residuals (as a measure of the difference between the shape of the MWD). The sum of the absolute residuals was then calculated and this was presented in Table S-6. Since the samples were measured in triplicate, they were also compared with their repeats using the same method. This resulted in an average sum residual of 6.46 with an SD of 2.47. Therefore, sample pairs yielding a residual less than 11.4 (6.46 + 2 SD) were considered to have SP-c MWDs that were statistically indistinguishable using the current method. Residual values of sample pairs corresponding to the range of 2 to 5 SD above the average within-sample variation were marked as 'most likely different'. For residual scores exceeding 5 SD, the sample pairs were considered to be 'clearly different'. Table 1 displays the results of such pair-wise comparison purely based on the NC PS-c MWDs of the twenty samples. From these results, the potential to differentiate SP samples based on NC PS-c MWD was studied by calculating the discrimination power (DP) using the following equation [31]:

$$DP(\%) = 100 \cdot \left[1 - \frac{2 \cdot m}{n(n-1)}\right] \quad (1)$$

Where m is the number of indistinguishable sample pairs and n is the total number of samples. For the current sample set, a DP of 90.53% was obtained based on the NC PS-c MWDs (m = 18 indistinguishable sample pairs out of 190 total pairs).

For the comparison of the additives present in the SPs, the average wt% for each additive was calculated from the triplicate measurements (see Table S-7). The SP additive profiles were found to enable a high degree of discrimination. Differentiation was assumed if the wt%s for at least one of the additives exceeded 2 SD between a sample pair. Only one sample pair could not be discriminated, resulting in a DP of 99.47%. Based on the sample set used in this study, from the NCFS SP database (different products and/or different suppliers, see Table S-8), the additive profile demonstrated to be a near-perfect discriminator, which explains why additive composition is the conventional method for SP differentiation.

Nevertheless, the MWD information may be of great forensic relevance when SP samples of the same brand and supplier are considered. It can be expected that additive profiles are carefully controlled and maintained within tight specifications by the manufacturer as this strongly affects important product properties such as burning behavior and storage stability. However, it is anticipated that the MWD of the NC will vary given the natural origin of the raw material cellulose. So possibly batches of the same product type can be distinguished based on of the variations in the MWD.

4. Conclusions

A novel on-line separation system for the characterization and comparison of SPs based on heart-cut 2D-LC was developed. The separation system enables forensic experts to relate the NC MWD to the additive formulation. In the first dimension, SEC was used to obtain the PS-calibrated MWD of the NC component. The first 9.5 min of the SEC separation were performed at a high flow rate after which the flow rate was lowered to enable the additive segment of the SEC chromatogram to be fractioned and diluted with water using a mixer. A dilution ratio of one part ^{1}D effluent to twenty parts water (1:20 v/v) resulted in effective trapping of the additives. For elution of the additives from the trap and subsequent focusing on the ^{2}D separation column, a volume of 300 µL mobile phase B proved to be necessary to elute the highly retained analytes from the trap column. To maintain sufficient dilution for focusing on the ^{2}D column, a dilution ratio of trap-column effluent to water of 3:20 (v/v) was used. These conditions resulted in complete analyte transfer of all additives prior to separation by gradient elution. Except for 2-NT and 3,4-DNT, all additives that were investigated in this study could be separated in the second dimension. Their UV absorbance is however slightly different and could be used for deconvolution.

By measuring two SPs tenfold over the course of a week, the system's performance was assessed and the error margins for both the NC PS-c MWD and additive profile were established. These margins were significantly lower than the variations observed for different SP samples as illustrated by the calculated DP values of 90.53 and 99.47%, respectively. The combination of the NC PS-c MWD and the additive composition of smokeless powders adds another orthogonal dimension for the forensic comparison of these types of propellants. Moreover, the developed 2D-LC method omitted the need for extensive sample preparation to selectively re-

move the NC fraction from the additives. Essentially, extraction of NC was performed in an on-line manner, resulting in an overall less labor-intensive and less error-prone method. Furthermore, the described modulation approach may be of interest to separations using different mechanisms (*e.g.* combining NPLC and RPLC). In the current state, the modulation interface is too slow for LC × LC implementation but with further research this might be a feasible solution.

Ultimately, MS-based detection could be added to the ^{2}D RPLC as an extra dimension for identifying the additive peaks and associated trace impurities. This will also solve the problem of the overlapping peaks of 2-NT and 3,4-DNT by being able to identify both based on their mass rather than the need to deconvolute their summed UV spectra. More additives could be added to the method as well as a calibration scheme based on multiple levels rather than the current one-point calibration. Furthermore, using a different ^{2}D column with larger internal diameter and more-retentive stationary phase may mitigate strong solvent effects. Moreover, there is still information in the NC fraction that was not yet extracted with the presented method, such as the degree of nitration and variation in nitration position. At this moment, it was not possible to use the UV data to estimate the degree of nitration and it may be interesting to have the degree of nitration as another orthogonal dimension for forensic comparison (*e.g.* by IR spectroscopy). Finally, no post-explosion material was investigated in this study, which would be interesting for future research. In future work, we intend to explore the options for post-explosion SP residue analysis with the current method and to investigate whether different SP batches from the same brand and type can be distinguished based on small changes in the MWD and the presence of impurities due to product degradation processes over time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Rick S. van den Hurk: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing – original draft, Visualization. **Noor Abdulhussain:** Conceptualization, Writing – review & editing. **Anouk S.A. van Beurden:** Methodology, Validation, Investigation, Formal analysis, Writing – original draft, Visualization. **Mabel E. Dekker:** Validation, Formal analysis. **Annemieke Hulsbergen:** Resources, Funding acquisition, Writing – review & editing. **Ron A.H. Peters:** Supervision, Funding acquisition, Writing – review & editing. **Bob W.J. Pirok:** Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing. **Arian C. van Asten:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Acknowledgements

This research is part of the PARADISE project (ENPPS.TA.019.001) and received funding from the Dutch Research Council (NWO) in the framework of the Science PPP Fund for the top sectors and from the Ministry of Economic Affairs of the Netherlands in the framework of the “PPS Toeslagregeling”. Prof. dr. Michael Sigman and dr. Mary Williams from the National Center for Forensic Science of the University of Central Florida (USA) kindly provided samples from the NCFS Smokeless Powders Database.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463072.

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