Contributions to Tobacco & Nicotine Research
formerly: Beiträge zur Tabakforschung International
Volume 32 · No. 1 · March 2023
DOI: 10.2478/cttr-2023-0004

Two Techniques for the Analysis of WS-3 With Potential Application to the Analysis of Other Cooling Agents *

by

Serban C. Moldoveanu and Robert O. Bussey

R.J. Reynolds Tobacco Co., Winston-Salem, NC, USA

SUMMARY

WS-3 (N-ethyl-*p*-menthane-3-carboxamide) is a compound used as a cooling agent for tobacco or nicotine products. Three other similar compounds WS-23 (N,2,3-trimethyl-2-propan-2-ylbutanamide), Evercool 180 (N-*p*-benzene-acetonitrile menthanecarboxamide), and Evercool 190 (N-(2-pyridine-2-yl)ethyl-3-*p*-menthancarboxamide) also have cooling properties and can be used as tobacco additives. A few previously reported methods for WS-3 analysis were rather complex, and the goal of present work was to develop a simple, rapid, and accurate procedure for simultaneous analysis of WS-3 and of the other three cooling compounds. For this purpose, a LC/MS procedure has been developed that requires 6-min runs. The method was calibrated to analyze solutions containing each analyte in the range of concentrations between 48.8 ng/mL to 12.5 µg/mL. The procedure is selective and shows excellent precision with typical RSD lower than 3%. In parallel to the LC/MS method, a GC/MS procedure was also developed mainly with the goal of having a proof of the accuracy for both methods. This GC separation requires 20-min runs. The GC/MS technique was calibrated to analyze solutions containing each analyte in the range of concentrations between 1.56 µg/mL to 125 µg/mL for WS-23 and WS-3, from 6.25 µg/mL to 125 µg/mL for Evercool 180, and from 3.13 µg/mL to 125 µg/mL for Evercool 190. The sensitivity of the LC/MS method is significantly higher than the GC/MS one, and for the analysis of traces of cooling agents the LC/MS method is recommended. The LC/MS method was applied for the analysis of WS-3 in tobacco, cigarette smoke, flavor solutions, and in nicotine pouches. No samples containing the other cooling agents were available. [Contrib. Tob. Nicotine Res. 32 (2023) 26–33]

KEYWORDS

WS-3, WS-23, Evercool 180, Evercool 190

ZUSAMMENFASSUNG

Bei WS-3 (N-Ethyl-*p*-Menthan-3-carboxamid) handelt es sich um eine Verbindung, die als Kühlmittel in Tabak oder Nikotinprodukten eingesetzt wird. Die drei weiteren ähnlichen Verbindungen WS-23 (N,2,3-Trimethyl-2-Propan-2-ylbutanamid), Evercool 180 (N-*p*-Benzol-Acetonitril-Menthancarboxamid) und Evercool 190 (N-(2-Pyridin-2-yl)ethyl-3-*p*-Menthancarboxamid) verfügen ebenfalls über Kühleigenschaften und können in Tabak als Zusatzstoffe verwendet werden. Die wenigen bereits vorgestellten Methoden zur Analyse von WS-3 waren eher komplex, und das Ziel der vorliegenden Arbeit war die Entwicklung eines einfachen, schnellen und präzisen Verfahrens zur zeitgleichen Analyse von WS-3 und den weiteren drei Kühlmitteln. Zu diesem Zweck wurde ein Flüssigchromatographie-Massenspektrometrie-Verfahren (LC/MS) mit einer Durchlaufzeit von 6 min entwickelt. Die Methode

*Received: 21st June 2022 – accepted: 9th March 2023

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0).
© 2023 Authors who publish with this journal retain all copyrights and agree to the terms of the above-mentioned CC BY-NC-SA 4.0 license.

wurde für die Analyse von Lösungen kalibriert, die die Analyten jeweils in Konzentrationen zwischen 48,8 ng/mL und 12,5 µg/mL enthielten. Das Verfahren war selektiv und wies eine ausgezeichnete Präzision mit einer typischen relativen Standardabweichung (RSD) von weniger als 3% auf. Parallel zur LC/MS-Methode wurde ebenfalls ein Gaschromatographie-Massenspektrometrie-Verfahren (GC/MS) mit dem Hauptziel entwickelt, einen Nachweis für die Genauigkeit beider Methoden zu erhalten. Diese GC-Trennung benötigt eine Durchlaufzeit von 20 min. Das GC/MS-Verfahren wurde für die Analyse von Lösungen kalibriert, die für WS-23 bzw. WS-3 die Analyten jeweils in Konzentrationen zwischen 1,56 µg/mL und 125 µg/mL enthielten, für Evercool 180 jeweils zwischen 6,25 µg/mL und 125 µg/mL und für Evercool 190 jeweils zwischen 3,13 µg/mL und 125 µg/mL. Die Empfindlichkeit der LC/MS-Methode war signifikant höher als die des GC/MS-Verfahrens, sodass für die Analyse von Kühlmittelspuren die LC/MS-Methode empfohlen wird. Die LC/MS-Methode wurde bisher für die Analyse von WS-3 in Tabak, Zigarettenrauch, Flüssigaromen für E-Zigaretten und Nikotinbeuteln angewandt. Proben, die die anderen Kühlmittel enthielten, standen nicht zur Verfügung. [Contrib. Tob. Nicotine Res. 32 (2023) 26–33]

RESUME

Le WS-3 (N-éthyl-*p*-menthane-3-carboxamide) est un composé utilisé en guise d'agent de refroidissement du tabac ou des produits contenant de la nicotine. Trois autres composés similaires, le WS-23 (N,2,3-triméthyl-2-propane-2-ylbutanamide), l'Evercool 180 (N-*p*-benzène-acétonitrile menthane carboxamide) et l'Evercool 190 (N-(2-pyridine-2-yl)éthyl-3-*p*-menthane carboxamide) présentent également des propriétés refroidissantes et se prêtent à une utilisation en guise d'additifs au tabac. Les quelques méthodes précédemment proposées et servant à analyser le WS-3 s'avéraient plutôt complexes et l'objet du présent projet fut de mettre au point un procédure précise, rapide et simple d'analyse simultanée du WS-3 et des trois autres agents de refroidissement. A ces fins, une procédure recourant à la chromatographie en phase liquide couplée à la spectrométrie de masse (CLP/SM) fut établie pour des durée d'essai requises de 6 minutes. La méthode fut calibrée pour l'analyse de solutions contenant chaque analyte dans des plages de concentration oscillant entre 48,8 ng/mL et 12,5 µg/mL. La procédure est sélective et affiche un excellent degré de précision avec un écart-type relatif typique inférieur à 3%. En parallèle de la méthode CLP/SM, un protocole basé sur une chromatographie en phase gazeuse couplée à une spectrométrie de masse (CPG/SM) fut également mise au point avec pour objectif principal d'obtenir une preuve de l'exactitude des deux méthodes. Cette séparation par CPG nécessite une durée d'essai de 20 min. La technique CPG/SM fut calibrée pour l'analyse de solutions contenant chaque analyte dans des plages de concentration oscillant entre 1,56 µg/mL to 125 µg/mL dans le cas des WS-23 et WS-3, entre 6,25 µg/ml et 125 µg/mL dans le cas de l'Evercool 180 et entre 3,13 µg/mL et 125 µg/mL dans le cas de l'Evercool 190. La sensibilité de la méthode CPL/SM s'avère significativement plus élevée que l'approche par CPG/SM; par conséquent, eu égard à l'analyse des traces des agents de refroidissement, la méthode CPL/SM est préconisée. La méthode CPL/SM fut appliquée pour l'analyse du WS-3 présent dans le tabac, la fumée de cigarette, les arômes liquides pour cigarettes électroniques et les sachets de nicotine. Aucun échantillon contenant les autres agents de refroidissement n'était disponible. [Contrib. Tob. Nicotine Res. 32 (2023) 26–33]

INTRODUCTION

WS-3 (N-Ethyl-*p*-menthane-3-carboxamide), CAS # 39711-79-0, *Mw* = 211.1, is a compound with cooling character used as additive to tobacco or to nicotine products (1). Other compounds with cooling effect such as WS-23 (N,2,3-trimethyl-2-propan-2-ylbutanamide), CAS # 51115-67-4, *Mw* = 171.1, Evercool 180 (N-*p*-benzeneacetonitrile menthanecarboxamide), CAS# 1187627-98-0, *Mw* = 298.2, and Evercool 190 (N-(2-pyridine-2-yl)ethyl-3-*p*-menthancarboxamide), CAS # 926913-58-8, *Mw* = 288.2 are also potential additives to tobacco and to nicotine products. The chemical structures of these compounds are indicated in Figure 1.

The analysis of WS-3 has been previously reported, e.g., using a GC-GC-TOFMS (2), a GC/MS method (3–5), and a LC-MS/MS method (6) and was reviewed in the literature (7). However, the reported methods for WS-3 analysis were rather complex, and the goal of present work was to develop a simple, rapid, and accurate procedure for simultaneous analysis of WS-3 and of the other three cooling compounds. This new procedure is based on an LC/MS procedure and the chromatographic run takes only 6 min. In parallel to the LC/MS method, a GC/MS procedure was also developed mainly with the goal of having a proof of the accuracy for both methods. The commonly used level of WS-3 in finished consumer products is between 5 µg/g and 1200 µg/g (8). A simple LC/MS method for the analysis of WS-3 is described in this report, the analysis allowing at the same time the analysis of WS-23, Evercool 180, and Evercool 190. The results of this method were compared with those of a GC/MS method that also allows the analysis of all four analytes. As the cooling compounds are present in some tobacco and nicotine products on the market, the new procedure will be of considerable utility for the analysis of these cooling agents in the products.

EXPERIMENTAL

Materials

The cooling agents were obtained from Vigon Intern. Inc. (East Stroudsburg, PA, USA). The HCOOH, methanol and nicotinamide (vitamin B3) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Water 18.2 mΩ/cm was obtained from a Barnstead Nanopure unit (Thermo Scientific Rockford, IL, USA). For the filtration of extracts, 0.45 µm PVDF filters were used (Whatman Autovial, GE Healthcare, Little Chalfont, UK). Scintillation vials of 20 mL with screw top caps and GC vials of 2 mL with screw top caps and septa were also utilized.

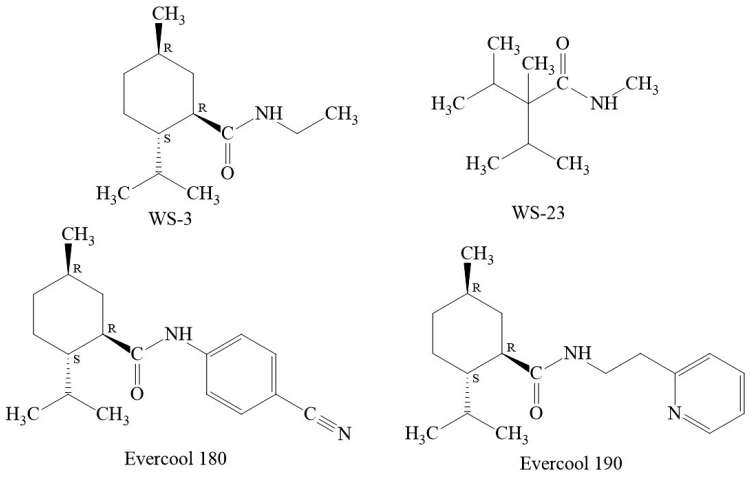


Figure 1. Chemical structures of four compounds with cooling properties.

Instrumentation for GC/MS analysis

The GC separation with MS detection was performed on a 7890B/5977A GC/MS instrument from Agilent (Wilmington, DE 19808, USA) equipped with a VF-1701ms column 30 m × 0.25 mm with 0.25 µm film from Agilent. Data interpretation was performed using MassHunter Qualitative Analysis B.07.00 and MassHunter Quantitative Analysis B.08.00. The extraction of samples was performed on a wrist action shaker (Burrell Co., Pittsburgh, PA, USA).

Instrumentation for LC/MS analysis

The HPLC separation with MS detection was performed on an Acquity I UPLC system that consisted of a binary pump, an autosampler with cooling capability, a QDa and a PDA detector from Waters (Milford, MA, USA). Data interpretation was performed using MassLynx version 4.1 program from Waters. The HPLC chromatographic separation was achieved on an Acquity UPLC® BEH C18 column 1.7 µm particles, 2.1 × 50 mm dimensions from Waters.

Standards preparation

Four solutions in methanol containing 125 µg/mL of WS-3, WS-23, Evercool 180, and Evercool 190, respectively, were prepared as stock solutions. Equal volumes from these solutions were mixed to obtain the Standard 1 that contained 25 µg/mL of each cooling agent. From this solution, 10 more standards were prepared, using repeated dilutions 50% previous standard 50% methanol, with the lowest standard containing 0.022 µg/mL. The standards were analyzed by both the GC/MS and the LC/MS procedures. For the GC/MS analysis only Standard 1 (25 µg/mL) to Standard 4 (1.56 µg/mL) were used for the calibration, and single-compounds solutions containing 125 µg/mL and 62.5 µg/mL coolant were also used. For the LC/MS analysis, only Standard 2 (12.5 µg/mL) to Standard 11 (0.024 µg/mL) were utilized. The GC/MS analysis could be used for more concentrated solutions of the analytes while the LC/MS procedure was more sensitive and could be used for diluted solutions. For the analysis, to 1 mL solution of standard was added 20 µL of a solution of an internal standard that consisted of 625 µg/mL nicotinamide in methanol to obtain in each standard a final concentration of 12.5 µg/mL I.S. (reported to 1 mL, not corrected to a final volume of 1.02 mL).

Sample preparation

Only various samples containing WS-3 cooling agent were available for analysis. The analyzed samples were extracted with methanol, the amount of sample and that of methanol were adjusted to obtain a final concentration in the range of calibration standards. The methanol extracts were analyzed directly and no cleanup was applied. The same extraction procedure used for the GC/MS analysis was used for the LC/MS analysis but for the LC/MS analysis the first extract was further diluted to bring the concentration in the range of calibration. In addition, two tobacco samples were also extracted on a Dionex-ASE-350 (Thermo-Fisher, Waltham, MA, USA) for proving the extraction efficiency.

For the tobacco samples, 400 mg tobacco were extracted with 10 mL methanol for 30 min on a wrist-action shaker. The solutions were filtered through 0.45 µm PVDF filters. For the GC/MS analysis the filtered solutions were directly used for analysis. For the LC/MS analysis a further dilution was necessary and 100 µL filtered tobacco extract was placed in a 2 mL vial and 900 µL of methanol were added to obtain 1 mL final solution for analysis (1:10 dilution).

For the nicotine pouches analysis, 400 mg pouch material was extracted with 10 mL methanol for 30 min. The solution was filtered through a 0.45 µL PVDF filter and further diluted 1:5 with methanol for LC/MS analysis. For the GC/MS, the 1:5 dilution is not necessary.

For the analysis of WS-3 in smoke, the total particulate matter (TPM) from three cigarettes was collected on a 44-mm Cambridge pad. Two smoking conditions were utilized, ISO (International Standard Organization) (9, 10) and HCA (Health Canada Intensive) (11). The smoking was performed at Eurofins Lancaster Lab at R.J. Reynolds Tobacco Co. The Cambridge pads containing the TPM were placed in 20-mL scintillation vial and extracted with 10 mL methanol for 30 min on a wrist action shaker. The extracts were filtered through a 0.45-µm PVDF filter and further diluted 1:10 for LC/MS analysis.

For the analysis of flavor solutions, 30 mg flavor solution were dissolved in 10 mL methanol. The obtained solution was further diluted 50 times by using 0.2 mL flavor solution and 9.8 mL methanol.

LC/MS analysis

The LC/MS analysis of the coolants with the separation performed on the Acquity UPLC® BEH C18 column in gradient conditions. Solution A for the gradient was 80% water, 20% methanol 0.1% HCOOH, and the solution was brought to pH 3.33 with a few drops of NH_4OH. Solution B was methanol. The flow rate of the mobile phase was 0.3 mL/min and the linear gradient conditions are indicated in Table 1.

Table 1. Gradient conditions for coolants HPLC separation.

Time (min)	A (%)	B (%)
0.0	95	5
0.5	95	5
2.0	40	60
4.0	40	60
5.0	95	5
6.0	95	5

The detection was performed in positive mode with the following ions monitored: m/z = 123.1 for nicotinamide (I.S.), m/z = 172.1 for WS-23, m/z = 212.2 for WS-3, m/z = 289.2 for Evercool 190 and m/z = 299.2 for Evercool 180. The QDa instrument had a cone voltage 15 V, gain = 1, temperature setting at 600 °C, capillary voltage 0.9 kV.
In the conditions for the LC described in Table 1 and for the QDa indicated above, a typical chromatogram for a standard containing 0.78 µg/mL of each analyte and 12.5 µg/mL internal standard is shown in Figure 2.
From the four coolants, WS-23, WS-3 and Evercool 180 are amides and their elution time is not much affected by the pH of the mobile phase. However, the pyridine moiety from the Evercool 190 makes the retention time of this compound sensitive to the mobile phase pH. For a mobile phase at pH 2.9, Evercool 190 elutes very close to WS-23, and for pH = 3.8 it coelutes with WS-3. For this reason, the pH of the mobile phase must be precisely controlled.
Based on a specific retention time for each analyte, of the specific mass detected by the MS detector and also on the specific conditions of detection (settings of the MS instrument) it can be concluded that the method has a very good selectivity.
The quantitation of coolants based on the LC/MS analysis was also performed using calibrations analyte as a function of peak areas but the experimental data were better fit by dependencies of the form

$$\boldsymbol{Y} = a\,\boldsymbol{X}^2 + b\,\boldsymbol{X} + c$$

where $\boldsymbol{Y}$ is the concentration of the cooling agent in mg/mL and $\boldsymbol{X}$ is the peak area of the specific ion of each analyte. The internal standard was used only to verify the reproducibility of the chromatographic process (was used as chromatographic standard) and was not applied for normalization of analyte peak areas. The use of normalized peak areas for quantitation did not lead to better precision of the quantitation. The parameters *a, b*, and *c* used for calibration for each cooling agent as well as the corresponding correlation coefficient R^2 are given in Table 2.

Table 2. The parameters *a*, *b*, and *c* used for calibration for each cooling agent as well as the corresponding correlation coefficient R^2.

Compound	*a*	*b*	*c*	R^2
WS-23	-3.91604E-14	5.64097E-06	-0.05549529	0.9987
WS-3	4.4729E-13	1.77042E-06	0.197442	0.9989
Evercool 190	-5.22754E-14	2.58027E-06	-0.02307681	0.9990
Evercool 180	1.61678E-12	6.22688E-06	0.007595738	0.9982

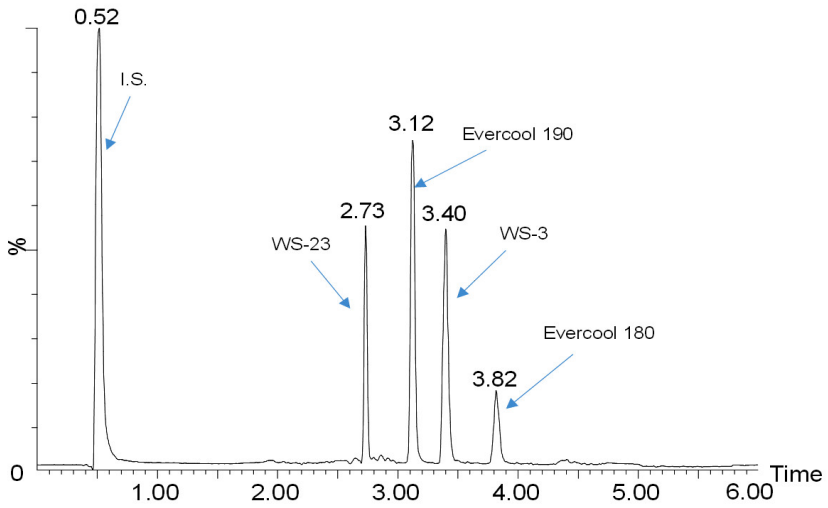


Figure 2. Typical LC/MS chromatogram for a standard containing 0.78 µg/mL of each analyte and 12.5 µg/mL internal standard (IS).

Table 3. The recovery of WS-3 from a tobacco sample spiked with three levels of analyte and the corresponding recovery levels.

Taken (mg/g)	Analyzed (mg/g)	Recovery (%)	RSD (%)
2.0	2.018	100.9	3.316
1.0	1.004	100.4	3.444
0.5	0.500	100.0	1.375

The calibrations were used to back-calculate the amount of cooling agent in the standards, and the limit of quantitation LOQ for each coolant was selected as equal with the lowest calibration standard that generated by back-calculation a result within 90–110%-range of the correct result for the average of two measurements with an RSD lower than 5–6%.

This procedure generated the value LOQ = 0.049 µg/mL for all four analytes. By considering that the limit of detection LOD is typically 1/3 LOQ, the technique can be considered as having LOD = 17 ng/mL.

In addition, the low RSD even for the lowest standard (lower than 5%) indicated that this procedure has a very good precision. For the analyzed samples, the RSD% (Tables 8–12) was also lower than 5% indicating a good precision for the whole procedure.

The extraction efficiency was evaluated only for WS-3 cooling agent. For the verification of extraction efficiency the tobacco samples of 1 g were spiked with a solution in propylene glycol of WS-3 containing 12.37% WS-3 by weight to obtain levels of WS-3 on tobacco of 0.2%, 0.1% and 0.05% WS-3. Each level was applied on one sample followed by triplicating the analysis. The results of the analysis of the spiked samples are indicated in Table 3.

The results from Table 3 indicate very good recovery of the WS-3. Further proof of efficiency of simple methanol extraction for the analysis of WS-3 was obtained by extracting 300 mg tobacco with 17.1 mL methanol in an ASE-350 accelerated solvent extractor, using a temperature of 100 °C with extraction for 10 min. The results for the level of WS-3 in two tobacco samples were within 97–103% range as for the simple methanol extraction.

The result for WS-3 is a good indication that the recovery of other cooling agents will be also good by the simple methanol extraction. However, the recovery for the other cooling agents was not tested.

The accuracy of the LC/MS procedure was proven by obtaining the correct results from the back-calculation of the levels of the analyzed cooling agents in the standard samples, from the excellent results obtained from the recovery data as indicated in Table 3, and also by obtaining the same values for WS-3 in the analyzed samples by the LC/MS procedure and by the GC/MS procedure as further shown in Tables 8 to 10.

GC/MS analysis

The GC/MS analysis of the coolants with the separation performed on the VF-1701ms column was performed in conditions indicated in Table 4.

Using the conditions described in Table 4, a typical chromatogram for a standard mix containing 25 µg/mL of each cooling agent and the internal standard at 12.5 µg/mL is shown in Figure 3.

For the quantitation of cooling agents using the GC/MS analysis, we used linear calibration curves of the type

$$\boldsymbol{Y} = a\,\boldsymbol{X} + b$$

where $\boldsymbol{Y}$ is the concentration of the cooling agent in µg/mL and $\boldsymbol{X}$ is the peak area of the specific extracted ion of each analyte.

The internal standard was used only to verify the integrity of the chromatogram (I.S. was used as chromatographic standard) and was not used for normalization of other peak areas since this procedure did not lead to better results. The ion used to generate the extracted peak in the chromatogram, the parameters *a* and *b* used for calibration for each cooling agent as well as the corresponding correlation coefficient R^2 are given in Table 5.

Table 4. Typical parameters for the GC/MS analysis.

Parameter	Description	Parameter	Description
Initial oven temperature	37 °C	Carrier gas	Helium
Initial time	1.0 min	Injection volume	1 µL
Oven ramp rate	30 °C/min	Flow mode	Constant flow
Oven temperature	70 °C	Flow rate	1.1 mL/min
Final time first ramp	0 min	Nominal initial pressure	7.95 psi
Oven ramp rate	20 °C/min	GC outlet	MSD
Oven final temperature	300 °C	MSD transfer line temperature	270 °C
Final time	6.4 min	Ion source temperature	230 °C
Total run time	20.0 min	Quadrupole temperature	150 °C
Inlet temperature	280 °C	MSD Gain	1
Inlet mode	Split	MSD solvent delay	5.0 min
Split ratio	10:1	Mass range	33–350 a.u.

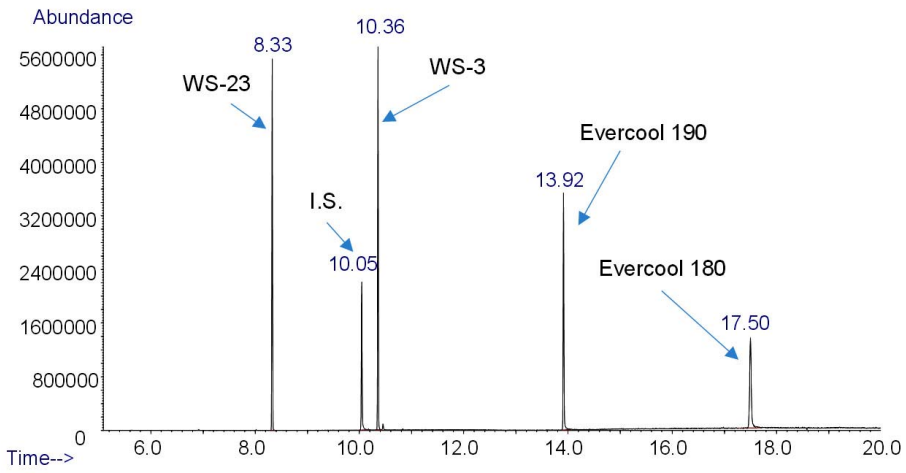


Figure 3. Typical GC/MS chromatogram of a standard mixture containing each coolant agent at 25 µg/mL and the internal standard (I.S.) at 12.5 µg/mL.

Table 5. Ion used to generate the extracted peak, the parameters *a* and *b* used for calibration for each cooling agent as well as the corresponding correlation coefficient R^2.

Compound	m/z for peak area	*a*	*b*	R^2
I.S. (nicotinamide)	122,1	-	-	-
WS-23	129,1	0	0,218801	0,9998
WS-3	211,2	0	-0,17814	0,9995
Evercool 190	288,1	0	2,604558	0,999
Evercool 180	298,1	0	3,44324	0,9986

Table 6. The LOQ values for the GC/MS method of analysis of cooling agents.

Compound	LOQ (µg/mL)
WS-23	1.56
WS-3	1.56
Evercool 190	3.13
Evercool 180	6.25

The calibrations were used to back-calculate the amount of cooling agent in the standards, and the LOQ for each coolant was selected as equal with the lowest calibration standard that generated by back-calculation a result within 90–110% range of the correct result for the average of two measurements with an RSD lower than 5–6%. As a result, the LOQ values for the analyzed cooling agents are those indicated in Table 6.

Based on the calibration range and using the same criterium as for establishing LOQ in LC/MS method (back-calculation results within 90–110% range of the correct value for the average of two measurements with an RSD % lower than 5–6%) the value for LOQ for the GC/MS method were those indicated in Table 6.

RESULTS AND DISCUSSION

Samples evaluated in the study

A number of samples containing WS-3 were analyzed by the LC/MS procedure, and a few of them also by the GC/MS procedure. The selection of the samples was made to include a variety of matrices such as tobacco, material from nicotine pouches, cigarette smoke and several flavors. The samples description is given in Table 7. The experimental cigarettes were made at R.J. Reynolds Tobacco Co. The commercial cigarettes were purchased from the market.

Results for tobacco from cigarettes

The levels of WS-3 in tobacco from cigarettes is indicated in Table 8. Each measurement was performed in triplicates.

Results for nicotine pouches

The levels of WS-3 in four nicotine pouches using the LC/MS analysis are indicated in Table 9. Each measurement was performed once and the RSD % is reported to different analyzed samples.

Sample (1) was also analyzed using the GC/MS procedure, also in triplicate. The levels of WS-3 in Sample (1) by GC/MS analysis are indicated in Table 10.

As indicated in Table 9, the LC/MS procedure for WS-3 analysis provides results with very good reproducibility and very close with the results for GC/MS analysis indicated in Table 10. This is a proof of the accuracy of the two methods.

Results for smoke analysis of experimental cigarette 1

The results for the levels of WS-3 in cigarette smoke are indicated in Table 11.

Table 7. Samples analyzed in this study.

Sample type	Material type	Analytical procedure
Experimental cigarette 1 [a]	Tobacco from cigarette	LC/MS, GC/MS
Experimental cigarette 2 [a]	Tobacco from cigarette	LC/MS
Experimental cigarette 3 [a]	Tobacco from cigarette	LC/MS
Experimental cigarette 4 [a]	Tobacco from cigarette	LC/MS
Commercial cigarette 1 [b]	Tobacco from cigarette	LC/MS
Commercial cigarette 2 [b]	Tobacco from cigarette	LC/MS
Nicotine pouch 1 [b]	Nicotine pouch	LC/MS, GC/MS
Nicotine pouch 2 [b]	Nicotine pouch	LC/MS
Nicotine pouch 3 [b]	Nicotine pouch	LC/MS
Nicotine pouch 4 [b]	Nicotine pouch	LC/MS
Experimental cigarette 1 [a]	Smoke ISO	LC/MS
Experimental cigarette 1 [a]	Smoke HCA	LC/MS
Flavor (1) to flavor (9) [a]	Flavor solution	LC/MS

[a] Cigarette manufactured at R.J. Reynolds Tobacco Co.
[b] Product available on the market

Table 8. Level of WS-3 per g of tobacco in several cigarettes.

Samples	Level of WS-3 (mg/g)	RSD (%)
Experimental cigarette 1 (LC/MS)	2.878	1.93
Experimental cigarette 1 (GC/MS)	2.991	4.93
Experimental cigarette 2	1.954	3.52
Experimental cigarette 3	< LOQ	
Experimental cigarette 4	1.484	0.02
Commercial cigarette 1	< LOQ	
Commercial cigarette 2	< LOQ	

Table 9. Results of WS-3 analysis by LC/MS in four analyzed nicotine pouches.

Samples	Level of WS-3 (mg/g)	RSD (%)
Sample 1 a	0.496	
Sample 1 b	0.513	
Sample 1 c	0.496	
Average for pouch 1	0.502	1.99
Sample 2 a	< LOQ	
Sample 2 b	< LOQ	
Sample 2 c	< LOQ	
Average for pouch 2	< LOQ	—
Sample 3 a	< LOQ	
Sample 3 b	< LOQ	
Sample 3 c	< LOQ	
Average for pouch 3	< LOQ	—
Sample 4 a	0.105	
Sample 4 b	0.103	
Sample 4 c	0.104	
Average for pouch 4	0.104	0.84

Table 10. Results of WS-3 analysis by GC/MS in four analyzed nicotine pouches.

Samples	Level of WS-3 (mg/g)	RSD (%)
Sample 1 a	0.514	
Sample 1 b	0.469	
Sample 1 c	0.490	
Average for pouch 1	0.491	4.59

Table 11. Results for the measurement of WS-3 in cigarette smoke.

Smoking	Level of WS-3 (mg/g)	RSD (%)
ISO	0.196	1.31
HCA	0.240	0.79

Table 12. Results for WS-3 levels in some flavor solutions.

Sample	WS-3 (mg/g)	RSD (%)
Flavor 1	125.69	4.33
Flavor 2	117.56	0.63
Flavor 3	123.72	2.74
Flavor 4	112.27	2.59
Flavor 5	119.50	3.82
Flavor 6	118.36	0.24
Flavor 7	121.13	2.95
Flavor 8	123.00	1.82
Flavor 9	113.89	1.84

Results for flavor solutions

For the analysis of flavor solutions, the problem was the large difference between the sample concentration in WS-3 (about 12%) and the range of calibration for the LC/MS analysis (48.8 ng/mL to 12.5 mg/mL). For this reason, the initial samples were diluted 16,666 times. The results for the WS-3 in the analyzed flavor solutions are indicated in Table 12.

The relatively low RSD% for the results from Table 12 prove the applicability of the LC/MS procedure for the analysis of flavor samples.

The application of the LC/MS analysis of WS-3 demonstrated the utility of the procedure for a variety of samples, showing that the matrix (tobacco, nicotine pouches, smoke, flavor solutions) does not affect adversely the analysis.

CONCLUSION

A few previously reported methods for WS-3 analysis were rather complex, and the goal of present work was to develop a simple, rapid, and accurate procedure for simultaneous analysis of WS-3 and of the other three cooling compounds WS-23, Evercool 180, and Evercool 190. This was realized by a new LC/MS method that runs the analysis in six minutes. The LC/MS method was used for the analysis of WS-3 on various samples, proving that the matrix of the sample does not affect adversely the analysis. A GC/MS method that can be successfully used for more concentrated samples was in parallel developed, and used to prove the accuracy of the two methods.

REFERENCES

1. Leffingwell, J.C. and D.G. Rowsell: Wilkinson Sword Cooling Compounds: From the Beginning to Now; Perfum. Flavor. 39 (2014) 34–43. Available at: https://www.researchgate.net/publication/260247091_Wilkinson_Sword_Cooling_Compounds_From_the_Beginning_to_Now (accessed March, 2023)
2. GC×GC-TOFMS of the Cooling Agent WS-3 in Mint Oils and Cinnamon Flavor, LECO Corporation, Form No. 203-821-290, 2008. Available at: https://gcms.cz/labrulez-bucket-strapi-h3hsga3/paper/PEG4D_COOLING_AGENT_IN_MINT_OILS_AND_CINNAMON_FLAVOR_203-821-290.pdf (accessed March, 2023)
3. Moldoveanu, S. and K. Kilby: Identification of Cooling Agents in Aerosols of an E-Cigarette From Unit Mass Resolution Spectra Enhanced to High Mass Accuracy; 62nd ASMS Conference, Baltimore, MD, USA, 2014, MP 470.
4. Reger, L., J. Moß, H. Hahn, and J. Hahn: Analysis of Menthol, Menthol-Like, and Other Tobacco Flavoring Compounds in Cigarettes and in Electrically Heated Tobacco Products; Beitr. Tabakforsch. Int. 28 (2018) 93–102. DOI: 10.2478/cttr-2018-0010
5. Jabba S.V., H.C. Erythropel, D.G. Torres, L.A. Delgado, J.G. Woodrow, P.T. Anastas, J.B. Zimmerman, and S.-E. Jordt: Synthetic Cooling Agents in US-marketed E-Cigarette Refill Liquids and Popular Disposable E-Cigarettes: Chemical Analysis and Risk Assessment; Nicot. Tob. Res. 24 (2022) 1037–1046. DOI: 10.1093/ntr/ntac046
6. Kondić, T., R. Singh, A. Elapavalore, and E. Schymanski: MassBank Record: LU130201; Available at: https://massbank.eu/MassBank/RecordDisplay?id=LU130201 (accessed March, 2023)
7. Leventhal, A.M., A.P. Tackett, L. Whitted, S.E. Jordt, and S.V. Jabba: Ice Flavours and Non-Menthol Synthetic Cooling Agents in E-Cigarette Products: A Review; Tob. Control (2022)1–9. DOI: 10.1136/tobaccocontrol-2021-057073
8. Leffingwell & Associates: Flavor-Base; 9th Ed. 2013.
9. International Organization for Standardization (ISO): ISO 3308: Routine Analytical Cigarette Smoking Machine – Definitions and Standard Conditions; Reference number ISO 3308:1991 (E) ISO, Geneva, Switzerland, 1991.
10. International Organization for Standardization (ISO): ISO 4387: Cigarette – Determination of Total and Nicotine Free Dry Particulate Matter Using a Routine Analytical Smoking Machine; Reference number ISO 4387:1991 (E), ISO, Geneva, Switzerland, 1991.
11. Canada Government Tobacco Act: Tobacco Reporting Regulations, SQR/2000-273; Registration June 26, 2000, Schedule 2: Official Method for Collecting Emission Data on Mainstream Smoke.

Corresponding author:

Serban C. Moldoveanu
R. J. Reynolds Tobacco Co.
Winston-Salem, NC, USA
E-mail: moldovs@rjrt.com