AUTOMATED EXTRACTION OF FREE BASE AND PROTONATED NICOTINE FROM E-LIQUIDS

APPLICATION NOTE AS-240

Authors

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Abstract

The determination of free-base and protonated nicotine in e-liquids is a requirement of the Tobacco Products Directive and is usually carried out via manual solvent extraction and gas chromatography analysis. This Application Note describes an automated extraction procedure, using the GERSTEL MPSrobotic platform, as well as automated preparation of calibration standards. The automated calibration preparation shows better linearity – 0.9997 vs. 0.9977 – than the manual procedure and the sample extraction compares favourably to the manual procedure. An additional benefit is the significant reduction in analyst time, with the only manual part of the process being the initial addition of the e-liquid samples to the extraction vials.

INTRODUCTION

The determination of free-base and protonated nicotine in e-liquids is a requirement of the Tobacco Products Directive, to accurately determine nicotine content and ensure that it is below the maximum threshold of 2% in total.

Manual preparation, particularly if sample numbers are high, can lead to bottlenecks that cause throughput challenges and depending on the skill of the analyst, there is a possibility data quality may be impacted. An automated method that can free up analyst time is a significant benefit. Additionally, the improvement in data quality that automation can bring, enables a "right first time" testing philosophy, despite the strain of multiple samples, and thus reduce the need for expensive retesting.

Alongside sample preparation, other benefits include the ability to automate preparation of calibration and system suitability standards – a time-consuming and laborious process that can have significant impact on the testing.

This Application Note demonstrates the automated extraction of both free-base and protonated nicotine, from e-liquids, along with automated preparation of the associated calibration standards. Comparison of the automated versus manually prepared calibration standards is presented.

EXPERIMENTAL

Instrumentation & GC Method

- GERSTEL Dual head MultiPurpose Sampler (MPS) Robotic/RoboticPro
- GERSTEL QuickMix
- Anatune CF200 Robotic centrifuge.
- Agilent 7890B with Flame Ionisation Detector

GC-FID method conditions:

Column: DB Wax 10m x 0.25mm x 0.5µm

Carrier Gas: Helium 0.8 ml/min

Injection: 1µL injection at 20:1 split

Inlet Temperature: 280°C inlet,

Detector: FID detector at 280°C (H_2 40 mL/min, Air 400 mL/min, make-up 15 mL/min)

Oven Program: 40°C (hold 1 min), 50°C/min to 240°C (hold 2 mins)

Figure 1: *GERSTEL MultiPurposeSampler (MPS) used for nicotine extraction.*

METHOD

Calibration Preparation - Manual

The Nicotine stock solution was prepared at 20 mg/mL in dichloromethane (DCM) with the internal standard solution (heptadecane) prepared at 2.5 mg/mL in DCM.

For the manual preparation, calibration standards are prepared, into volumetric flasks as detailed in Table 1, prior to aliquoting into 2 mL GC vials. The internal standard volume is 1 mL in all cases.

Sample Preparation - Manual

Samples of e-liquid are manually prepared for analysis using the following procedure –

- Weigh 0.1g of e-liquid into 20mL headspace vial.
- Add 4 mL UP water and mix.
- Add 4 mL of internal standard/DCM solution and close vial.
- Extract for 30 minutes on disc rotator.
- Allow partitioning of layers and remove DCM layer.
- Add 2 mL 1N NaOH to water and mix.
- Add 4 mL of internal standard/DCM solution and close vial.
- Extract for 30 minutes on disc rotator.
- Allow partitioning of layers (approximately 30 minutes) and remove aliquot of DCM.
- Analyse samples.

Standard	Volume of Stock / µL	Final Volume / mL	Final Conc. of Nicotine / µg/mL	Final Conc. Of Int. Std. / µg/mL
1	0	10	0	250
2	5	10	10	250
3	50	10	100	250
4	150	10	300	250
5	250	10	500	250
6	500	10	1000	250
7	1250	10	2500	250
8	2500	10	5000	250
9	3750	10	7500	250

Table 1: *Manually prepared calibration standards*

Calibration Preparation - Automated

For the automated preparation, the calibration standards were directly prepared into 2 mL GC vials, as detailed in Table 2 below. Due to the low levels for standards 2 and 3, a separate stock solution, diluted ten-fold, was used for these two levels. The internal standard volume is 0.1 mL in all cases.

Sample Preparation

For the automated preparation, the following procedure was developed, which is completely automated except for the initial addition of e-liquid into the 10 mL headspace vial. Figure 2 shows an example time schedule for the preparation of 6 replicates of e-liquid.

- Weigh 0.1g of e-liquid into 10mL headspace vial and close vial.
- Add 4 mL of UP water and mix for 1 minute using QuickMix.
- Add 4 mL of internal standard/DCM solution.
- Extract for 5 minutes on QuickMix.
- Centrifuge for 1 minute to force partition and remove 1.5 mL of DCM layer to 2 mL vial.
- Remove 2 mL of water layer to new 10 mL capped headspace vial.
- Add 2 mL of 1N NaOH solution.
- Add 4 mL of internal standard/DCM solution.
- Extract for 5 minutes on QuickMix.
- Remove 1.5 mL of DCM layer to 2 mL vial.
- Analyse samples.

Standard	Volume of Stock / µL	Volume of DCM / mL	Final Volume / mL	Final Conc. of Nicotine / µg/mL	Final Conc. Of Int. Std. / µg/mL
1	0	0.900	1	0	250
2	5	0.985	1	10	250
3	50	0.850	1	100	250
4	15	0.885	1	300	250
5	25	0.875	1	500	250
6	50	0.850	1	1000	250
7	125	0.775	1	2500	250
8	250	0.650	1	5000	250
9	375	0.525	1	7500	250

Table 2: *Automated preparation of calibration standards (note – the shaded rows correspond to the low level stock preparation).*

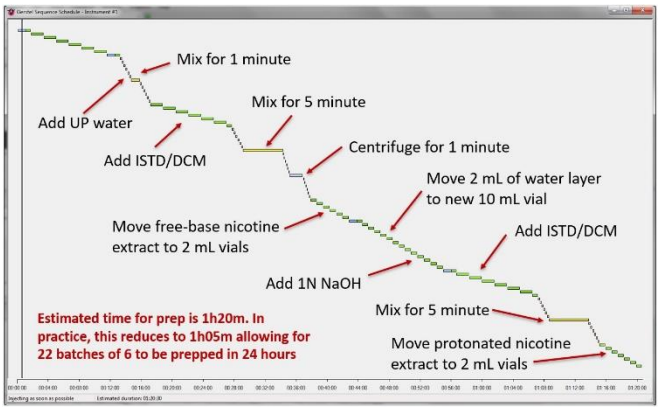

Figure 2: *PrepSequence schedule of automated nicotine extraction from e-liquids.*

GC-FID analysis

Analysis of both calibration standards and samples was carried out using GC-FID. The method is detailed in the experimental section above. Two sets of calibrations were analysed – a manually prepared set supplied externally and an automated set prepared in-house at Anatune. A single set of five replicate samples of e-liquid was analysed, against the calibration set prepared in-house. No externally prepared samples of e-liquids were supplied.

RESULTS AND DISCUSSION

Calibrations

Figure 3 shows an example chromatogram of calibration standard 5, with the internal standard eluting before the nicotine.

Integration data for both the manually prepared and automated calibration sets are shown in Tables 3 and 4. The variability in internal standard responses (RSD = 20.2) for the manually prepared calibrations are significantly greater than for the automated preparation (RSD = 5.0). As the manually prepared samples were prepared off-site and sent to the laboratory pre-vialled, some differential evaporation of the solvent may have occurred, leading to the wide variability seen. However, it is clear from the correlation coefficient (R^2), that the linearity of the automated preparation is superior – R^2 = 0.9997 vs. R^2 = 0.9977.

Plots of the data presented in Tables 3 and 4 are shown below (figures 4 and 5).

Table 3: *Integration data for manually prepared calibration standards.*

Nicotine Concentration / µg/mL	Nicotine Response	Internal Standard Response	Response Ratio
0	0	5890346	0
10	161156	8541836	0.018867
100	2329112	7246879	0.321395
300	7851882	8307804	0.945121
500	10465100	6835653	1.530958
1000	23078190	8180504	2.821121
2500	51705051	7995898	6.466447
5000	62411797	5536170	11.27346
7500	71811080	4167737	17.23023

Internal standard response (RSD) = 20.2
Linearity (R^2) = 0.9977

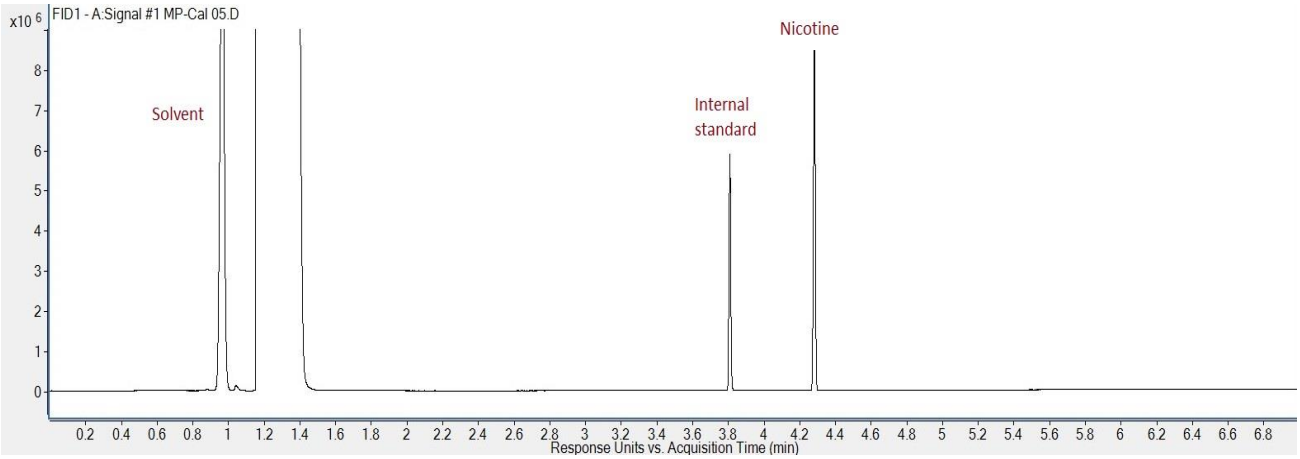

Figure 3: *Example chromatogram for calibration standard 5 (automated).*

Table 4: *Integration data for automated calibration standards.*

Nicotine Concentration / µg/mL	Nicotine Response	Internal Standard Response	Response Ratio
0	2592	4016760	0.000645
10	184337	4109488	0.044856
100	1535686	3852378	0.398633
300	3619384	3935990	0.919561
500	6316083	3990021	1.58297
1000	12363895	3823260	3.233862
2500	33550618	4137085	8.109724
5000	59058940	3682305	16.03858
7500	86390315	3488492	24.76437

Internal standard response (RSD) = 5.0
Linearity (R^2) = 0.9997

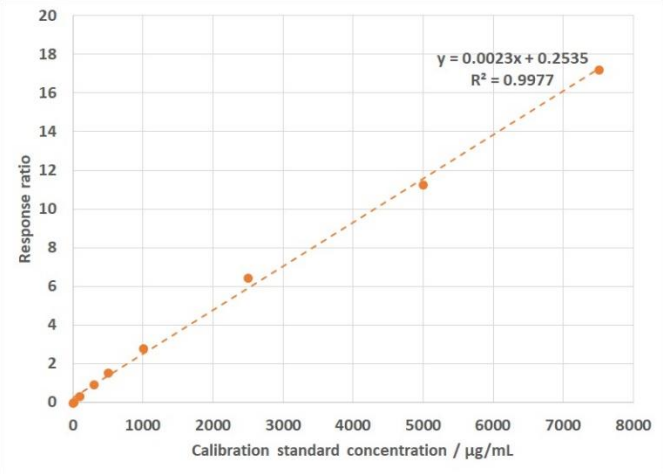

Figure 4: *Linearity plot for manually prepared calibration standards.*

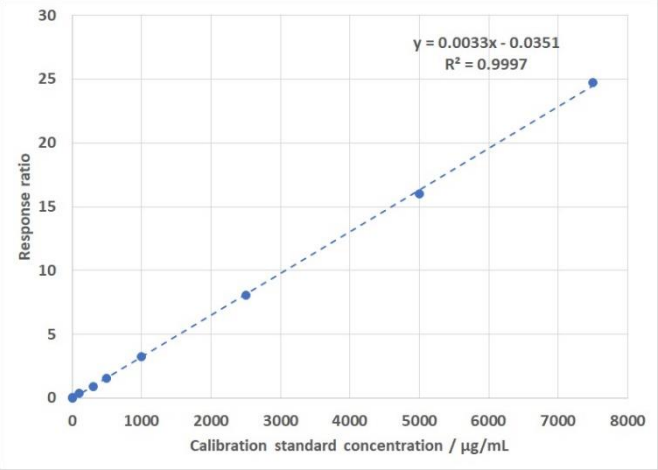

Figure 5: *Linearity plot for automated calibration standards.*

Sample analysis

Figures 6a and b show overlaid chromatograms for five replicate extractions for free-base and protonated nicotine, respectively, whilst Table 5 shows the integration results for this data. Table 6 details the amount of nicotine, in mg/g of e-liquid, in the samples, calculated from the calibration curve shown in figure 5 and is shown graphically in figure 7.

The data clearly demonstrates good reproducibility between the extracts, both with the initial free base extraction and the subsequent protonated extraction. Further, the total nicotine content from Table 6 is highly consistent, resulting in an RSD of 0.6%.

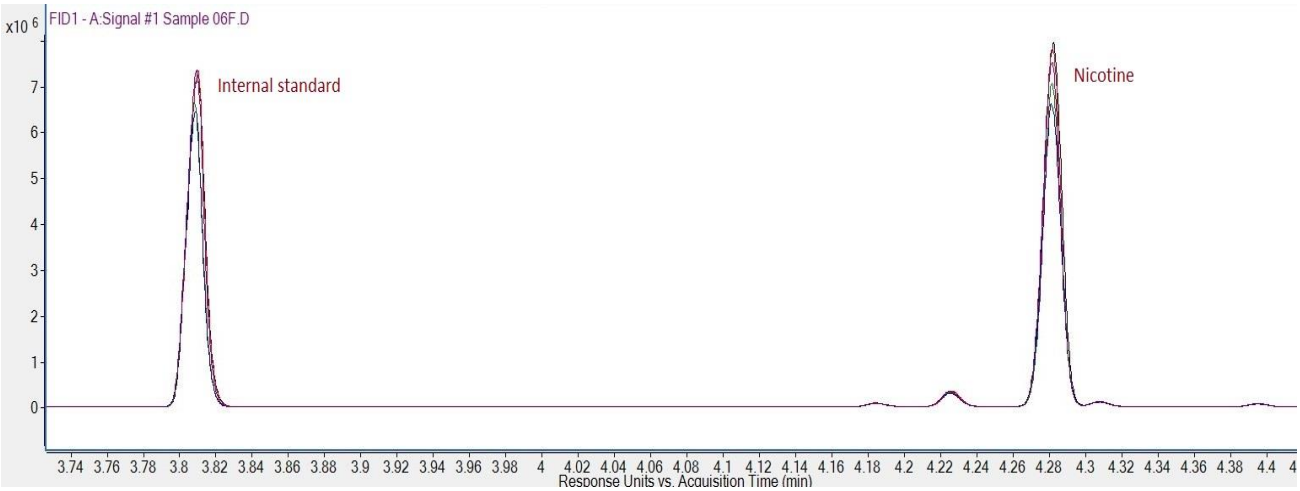

Figure 6a: *Overlaid chromatograms for extraction of free-base nicotine from the e-liquid (n=5).*

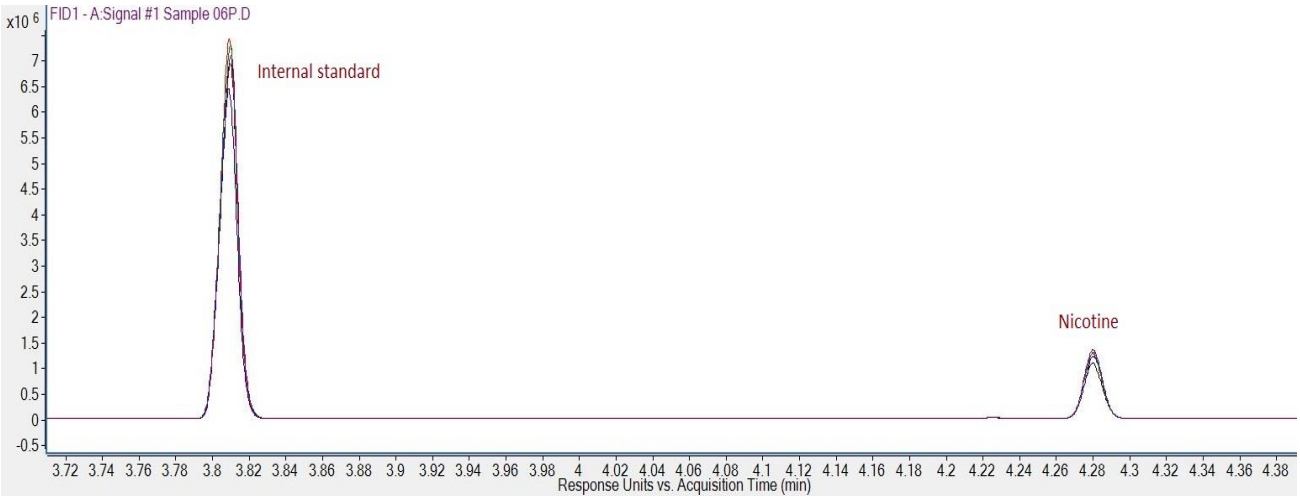

Figure 6b: *Overlaid chromatograms for extraction of protonated nicotine from the e-liquid (n=5).*

Table 5: *Integration data for both the free-base and protonated nicotine extraction (n=5).*

Sample	Nicotine (Free base)	Int. Std. (Free base)	Res. ratio (Free base)	Nicotine (Proton.)	Int. Std. (Proton.)	Res. ratio (Proton.)
Rep 1	7895290	7088267	1.113853	1071762	6914440	0.155003
Rep 2	7782192	7312800	1.064188	1274079	7383634	0.172554
Rep 3	7034854	6634588	1.06033	1253786	7261204	0.172669
Rep 4	6578567	6426373	1.023683	1202350	6434933	0.186847
Rep 5	7477899	7326398	1.020679	1336941	7067736	0.189161
Mean	7353760	6957685	1.056547	1227784	7012389	0.175247
%RSD	6.6	5.2	3.2	7.3	4.7	7.0

Table 6: *Free-base, protonated and total nicotine content in analysed e-liquid (n=5).*

Sample	Free base Nicotine / mg/g in e-liquid	Protonated Nicotine / mg/g in e-liquid	Total Nicotine / mg/g in e-liquid
Rep 1	14.03	4.64	18.67
Rep 2	13.42	5.07	18.49
Rep 3	13.37	5.07	18.44
Rep 4	12.92	5.42	18.34
Rep 5	12.89	5.47	18.36
Mean	13.33	5.13	18.46
%RSD	3.1	5.8	0.6

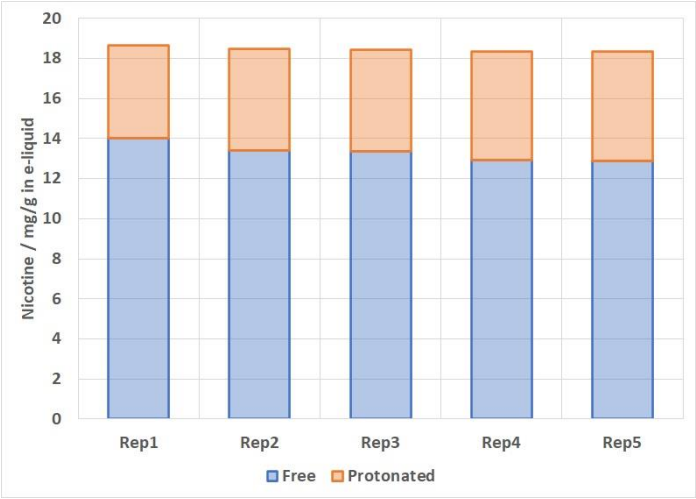

Figure 7: *Plot of data presented in Table 6*.

DISCUSSION

This report demonstrates that both the calibration standard preparation and e-liquid extraction for free-base and protonated nicotine can be fully automated on the GERSTEL MPS RoboticPro system. The linearity for the instrument prepared calibrations showed a higher R^2 value than those prepared manually (0.9997 vs 0.9977). It can be seen in both Table 6 and figure 7 that the total nicotine content shows very good repeatability – 0.6% RSD.

In addition to the improved accuracy and precision that automated standard and sample preparation brings, there are benefits to be gained from increased laboratory staff productivity. Once the stock standards and the initial e-liquid samples have been prepared, there are no further inputs required from the user.

Also, when comparing the automated methods to the manual methods – particularly the calibration preparation – the volume of solvents and standards used is reduced. This has three benefits, reduced purchase costs, reduced volumes for disposal and reduced exposure of potentially harmful chemicals for the analyst. Indeed, it may be possible, given the improving sensitivities of newer instruments, to further reduce sample and standard volumes.

Finally, the modular nature of the GERSTEL MPS system allows for other, complex procedures to be automated, beyond those shown in this Application Note.

To discuss implementing this application for your e-liquid sample and standard preparation, contact us and we will be delighted to work with you from conception to method transfer into your laboratory.