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AN OVERVIEW ON ANALYTICAL PROFILE OF VARENICLINE

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Abstract:
Varenicline is a medicinal agent used to treat smoking addiction. Varenicline is first permitted nicotinic receptor partial agonist. Varenicline is an orally administered α_4 andβ_2 nicotinic acetylcholine receptor partial agonist. The present study evaluates the various approaches for analysis of VRC in bulk drug as well as formulated products. A short review characterize the collection and discussion of about more than 23 analytical methods which includes HPLC, UPLC, capillary zone electrophoresis, liquid-chromatography mass-spectroscopy methods (LC-MS)and UV-Spectrophotometry methods implemented for investigation of VRC in pharmaceutical matrix. The proposed review article summarizes different reported methods developed to help researchers in choosing significant parameters for new analytical method of Varenicline.

Keywords: *Varenicline; method development; HPLC; capillary electrophoresis*

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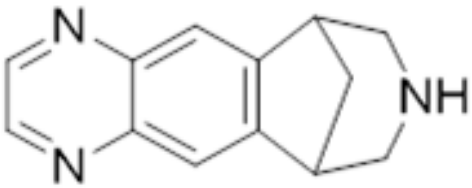
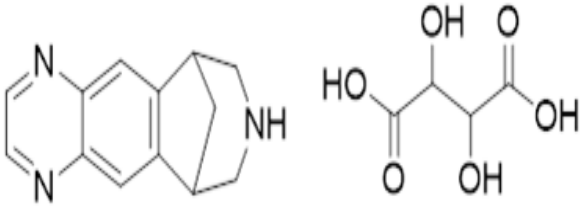
INTRODUCTION:

Varenicline (VRC) is chemically know as 7,8,9,10-Tetrahydro-6,10-methano-6*H*-pyrazino(2,3-h) (3) benzazepine was shown in **Figure 1**. The molecular weight of VRC is 211.26 g/mol [1].It is cream-colored solid with melting point of VRC in the range of 137-139ºC; itis highly soluble in water. VRC is an orally administered $\alpha_4\beta_2$nicotinic acetylcholine receptor partial agonist. For utilize since an aid to smoking cessation therapy [1-3].



Varenicline



Varenicline Tartrate

Pharmacology

In vitro VRC showed high affinity for the rat cortex$\alpha_4\beta_2$ nicotine receptor in radio-ligand displacement binding assays with a Ki value of0.17nM. It is supposed that the nicotinic $\alpha_4\beta_2$receptor mediates dopamine release in the nucleus accumbens and thus is involved in the inspiration effects of smoking. Both VRC and nicotine reversibly bind to the same receptor binding site. Considering the 15-fold greater affinity of VRC for the $\alpha_4\beta_2$ nicotinic acetylcholine receptor as compared with nicotine, very high nicotine brain concentrations would be necessary to completely displace VRC and produce nicotine rewarding in the subject. In vivo VRC only partly initiates the mesolimbic dopamine system, in comparison to the activation induced by nicotine. VRC released 3H-dopamine from rat striatal slices with a maximal response of 51% (relative to the release evoked by nicotine) at 1mM, indicating that VRC acts as a partial agonist at the nicotinic acetylcholine receptor. Additionally, 10 mM VRC reduced the response induced by nicotine by 53%, down to levels induced by VRC [4].

Pharmacokinetics

Absorption

The absorption of VRC was primarily complete after oral administration and systemic availability was high. Maximal plasma concentrations of VRC are reached within 3–4h and steady-state concentrations take place within 4 days. VRC has a half-life of 24h, and its bioavailability is not affected by food or time of administration. VRCexhibits a linear pharmacokinetics and low plasma protein binding (20%) [5, 6].

Distribution

The tissue distribution of VRC was in the majority of tissues, VRC distributed into all tissues except the lens and vitreous humor. Still, VRC showed a high affinity for ocular tissues, and there were no apparent gender differences with respect to tissue distribution. VRC displays affinity for melanin containing tissues [7].

Drug metabolism and excretion

Most of orally ingested VRC is excreted unchanged in urine. However, there are two inactive metabolites that are observed in human urine, namely 2-hydroxyvarenicline and VRC N-carbamoyl glucuronide

VRC was largely evacuated as unaffected drug substance, and represented the vast majority of drug-related material in circulation, although some metabolites were detected however, in several instances, the amounts of metabolite were inadequate, preventing definitive structural assignment, owing to the small amount of metabolism. Occurring for VRC and the low doses administered [7].

Analytical account of VRC [8-23]

The extensive literature survey revealed several analytical techniques viz UV/Vis-Spectrophotometry, HPLC and LC-MS for the determination of VRC in bulk and pharmaceutical formulations. The reported methods describe the estimation of VRC in various dosage forms as single constituent.Thedifferent analytical methods were implemented for estimation of VRC are shown in **Figure 2**.

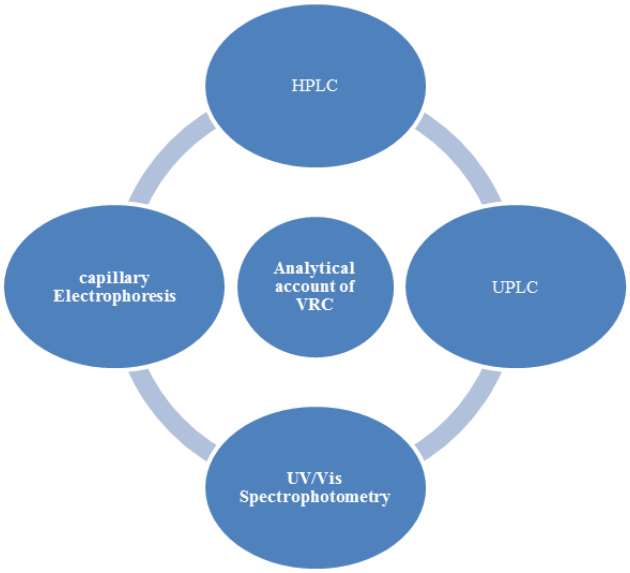


Figure 2: Analytical methods of VRC

Accounts Capillary zone electrophoresis Method for Determination of VRC

Alhazmi, H.A.et al. reported a fast, sensitive and selective method for the detection and quantification of VRC in tablet dosage form was developed using capillary zone electrophoresis separation with DAD detector. The used capillary was uncoated standard bare fused-silica with 100 µm I.D, 40 cm total length and 31.5 cm effective length. The electrophoretic separations were carried out in 50 mM sodium phosphate buffer (pH 2.5) under normal mode (30 kV). Samples were filtered through a 0.22µm CME membrane filter and then injected hydrodynamically at a pressure of 50 mbar for 10 s. The calibration curve of VRC was linear (r=0.998) over the concentration range 1–16 µg/ml. The LOD and LOQ values were 0.2 and 0.6 µg/ml, respectively. Finally, the method proved to be suitable for quality control studies [8].

Celebier, M. el al. reported in this study, analyses were carried out on a fused-silica capillary (i.d. 50.0 µm, total length 48.5 cm, and effective length 40.0 cm) in normal mode by applying a voltage of 20 kV. Sample injections were made in hydrodynamic mode over 7 s under a pressure of 50 mbar. The capillary temperature was set at 35 ºC and the detection was performed at a wavelength of 205 nm. The background electrolyte was 40 mM citrate buffer at pH 6.0 and the internal standard was labetalol hydrochloride. The total analysis time was shorter than 5 minutes. The method was validated according to the International Conference on Harmonization (ICH) guidelines, and the method was found to be linear, precise, accurate, specific, robust, and rugged. The linearity range was found to be 1.0–60.0 µg/ml and the limit of detection and quantitation were 0.5 and 1.0 µg ml–1, respectively. The developed method was proposed for quality-control laboratories [9].

Piestansky, J. et al. reported a new highly advanced analytical approach, based on two-dimensional column coupled CE (ITP-CZE) hyphenated with tandem mass spectrometry (MS/MS, here triple quadrupole, QqQ) was developed, evaluated and applied in biomedical field in the present work. Capillary isotachophoresis (ITP) coupled on-line with capillary zone electrophoresis (CZE) used in hydrodynamically closed separation system was favorable for increasing the sample load capacity, increasing the analyte concentration, and removing the deteriorative highly conductive major matrix constituents. These factors considerably reduced the concentration limits of detection (cLOD) and external sample preparation (comparing to single column

CZE), and, by that, provided favorable conditions for the mass spectrometry (enhanced signal to noise ratio, reproducibility of measurements, working life of MS). Here, the CZE–ESI combination provided more effective interfacing than ITP–ESI resulting in both a higher obtainable intensity of MS detection signal of the analyte as well as reproducibility of measurements of the analyte's peak area. The optimized ITP-CZE–ESI- QqQ method was successfully evaluated as for its performance parameters (LOD, LOQ, linearity, precision, recovery/accuracy) and applied for the direct identification and ultratrace (pg mL_1) determination of VRC and, in addition, identification of its targeted metabolite, 2-hydroxy-varenicline, in unpre- treated/diluted human urine. This application example demonstrated the real analytical potential of this new analytical approach and, at the same time, served as currently the most effective routine clinical method for VRC [10].

Piestansky, J. et al. reported two capillary electrophoresis methods for monitoring renally excreted VRC, a highly effective drug prescribed for smoking cessation, in human urine were developed and compared. A method combining capillary electrophoresis with mass spectrometry was proposed for the fast analysis of VRC(analysis time up to 7 min). Here, mass spectrometry was a prerequisite for achieving high sensitivity and selectivity of the analysis suitable for the quantification of a 15 ng.mL-1 level of VRC in un-pretreated urine matrices. An alternative approach, two-dimensional (column-coupled) capillary electrophoresis with enhanced sample load capacity and ultraviolet detection, was proposed as a low-cost alternative to capillary electrophoresis with mass spectrometry. The isotachophoresis on-line sample treatment included simple elimination of the major matrix constituents and stacking of the sample in a large volume so that threefold lower quantitation limits could be easily achieved in comparison to the capillary electrophoresis with mass spectrometry. On the other hand, longer analysis time (ca. 4.5-fold), and more complex electrolyte system in the coupled zone electrophoresis step (including two additives enhancing separation selectivity, i.e. isopropanol and cyclodextrin) were prerequisite for the complete separation of VRC from the sample matrix. Anyway, both the developed methods were validated according to the Food and Drug Administration guideline showing favorable performance parameters, suitable for their routine biomedical use [11].

Stolz, A., et al. reported capillary electrophoresis (CE) offers fast and high-resolution separation of charged analytes from small injection volumes. Coupled to mass spectrometry (MS), it represents a powerful analytical technique providing (exact) mass information and enables molecular characterization based on fragmentation. Although hyphenation of CE and MS is not straightforward, much emphasis has been placed on enabling efficient ionization and user-friendly coupling. Though several interfaces are now commercially available, research on more efficient and robust interfacing with nano-electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI) and inductively coupled plasma mass spectrometry (ICP) continues with considerable results. At the same time, CE-MS has been used in many fields, predominantly for the analysis of proteins, peptides and metabolites. This review belongs to a series of regularly published articles, summarizing 248 articles covering the time between June 2016 and May 2018. Latest developments on hyphenation of CE with MS as well as instrumental developments such as two-dimensional separation systems with MS detection are mentioned. Furthermore, applications of various CE-modes including capillary zone electrophoresis (CZE), nonaqueous capillary electrophoresis (NACE), capillary gel electrophoresis (CGE) and capillary isoelectric focusing (CIEF) coupled to MS in biological, pharmaceutical and biological research are summarized [12].

Beutner, A. et al. reported for the identification and quantification of analytes in complex samples, highly selective analytical strategies are required. The selectivity of single separation techniques such as gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE) with common detection principles can be enhanced hyphenating orthogonal separation techniques but also complementary detection systems. In this review, two-dimensional systems containing CE in at least one dimension are reviewed, namely LC-CE or 2D CE systems. Particular attention is paid to the aspect of selectivity enhancement due to the orthogonality of the different separation mechanisms. As an alternative concept, dual detection approaches are reviewed using the common detectors of CE such as UV/VIS, laser-induced fluorescence, capacitively coupled contactless conductivity (C4D), electrochemical ldetection, and mass spectrometry. Special emphasis is given to dual detection system simple menting the highly flexible C4D as one detection component. Selectivity enhancement can be achieved in case of

complementarity of the different detection techniques [13].

High-Performance Liquid-Chromatography (HPLC) Methods for VRC

Table 1: HPLC methods on VRC

Sr. No	Drug	Column	Mobile Phase	Detector	Wavelength (nm)	Retention Time (min)	Flow rate (ml/ min)	Ref.
1	VRC Tartrate	Kromasil C18 column (250 mm× 4.6 mm) Inertsil ODS 3V (150 mm× 4.6 mm)	Methanol:Potassium dihydrogen orthophosphate buffer pH 3 (50:50, v/v)	UV Detector	237	2.9	0.6	14
2	VRC	Zorbax Eclipse XDB-C8 column (150 mm× 4.6 mm)	Acetonitrile: potassium dihydrogen phosphate buffer (10:90,v/v)	PDA Detector	235	6.0	1.0	15
3	VRC	Inertsil ODS 3V (150 mm× 4.6 mm)	0.1% trifluroaceticacid,methanol&acetonitrile(8:1:1, v/v)	UV Detector	235	4.9	1.0	16
4	VRC	Ristek –Ultra® C18 column (100mm x 2.1 mm, 5 µm).	Buffer mixture (1.2% potassium dihydrogen phosphate and 0.08% octane sulphonic acid): Acetonitrile pH (5.0). (86: 14, v/v), pH (5.0).	DAD Detector	235	-	1.0	17
		Waters C18 column (150mm x 4.6 mm, 5µm)	Methanol - Distilled water (70: 30, v/v).	Fluorescence Detector	474	-	1.0	
5	VRC	Atlantis dC18 column (250mmx4.6mm)	Phosphate buffer with pH 3.0 and Acetonitrile (80:20, v/v)	PDA Detector	235	-	1.0	18
6	VRC	Chromolith Performance RP18e column (100 ×mm 4.6 mm)	Sodium benzoate (0.5 mmol/l) trifluoroacetic acid (20 mmol/l) (55:45, v/v)	DAD Detector	320	2.5	1.2	19

Channabasavaraj k. pet al. reported a reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of VRC tartrate in bulk and tablet using UV detector. Gradient chromatography was performed on a C-18 column, with a mobile phase composed by Methanol: Potassium dihydrogen orthophosphate buffer pH 3 (50:50, v/v), at flow rate of 0.6 ml/min using UV detection at 237 nm. The retention time for VRC tartrate was found to be 2.966 min. Linearity of the method was found to be 10 to 50 µg/ml, with the regression coefficient of 0.9999. This method was validated according to ICH guidelines. The intra-day and inter day percentage relative standard deviation (RSD) was found 0.327 and 0.147 respectively. The proposed method was successfully applied for the quantitative determination of VRC tartrate in tablet formulations [14].

Adnan A Kadi et al. reported a simple, sensitive and accurate stability-indicating HPLC method has been developed and validated for determination of VRC in its bulk form and pharmaceutical tablets. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C8 column (150 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature) by a mobile phase consisted of acetonitrile and 50 mM potassium dihydrogen phosphate buffer (10:90, v/v) with apparent pH of 3.5 ± 0.1 and a flow rate of 1.0 ml/min. The detection wavelength was set at 235 nm. VRC was subjected to different accelerated stress conditions. The degradation products, when any, were well resolved from the pure drug with significantly different retention time values. The method was linear (r = 0.9998) at a concentration range of 2 - 14 µg/ml. The limit of detection and limit of quantitation were 0.38 and 1.11 µg/ml, respectively. The intra- and inter-assay precisions were satisfactory; the relative standard deviations did not exceed 2%. The accuracy of the method was proved; the mean recovery of VRC was 100.10 ± 1.08%. The proposed method has high throughput as the analysis involved short run-time (~ 6 min). The method met the ICH/FDA regulatory requirements. The proposed method was successfully applied for the determination of VRC in bulk and tablets with acceptable accuracy and precisions; the label claim percentages were 99.65 ± 0.32%. The results demonstrated that the method would have a great value when applied in quality control and stability studies for VRC [15].

Prakash Katakam et al. reported VRC is suggested for use m smoking cessation and very few methods are reported for the determination of VRC. and A novel, simple, accurate, precise and stability indicating RP-HPLC method has been developed and validated for the assay of VRC in pharmaceutical formulations. Separation was achieved within 10 min with required asymmetry, accuracy and precision thus enabling the utility of the method for routine analysis. Chromatographic separation was achieved on a Inertsil ODS 3V5 um, mm using a mobile phase consisting of 0.1% tri fluroacetic acid, methanol and acetonitrile in the ratio of 8:1:1 at a flow rate of 1.0 ml, per min The detection was made at 235 nm. The retention time of VRC is 4.9 min. The method was validated and demonstrated good linearity, precision, accuracy and specificity in compliance with the regulatory requirements [16].

Ramzia I. El-Bagary et al. reported two simple, sensitive, rapid, and stability- indicating liquid chromatographic (LC) methods have been developed for the determination of VRC tartrate. They comprised the determination of VRC in the presence of its oxidative degradates and related impurity (N-formylVRC) (NFV). The first method was a LC with diode array detection (DAD) at 235 nm using Ristek – Ultra® C18 column (100 mm x 2.1 mm, 5 µm). Isocratic elution of VRC was employed using a mobile phase consisting of buffer mixture (1.2% potassium dihydrogen phosphate and 0.08% octane sulphonic acid): acetonitrile (86: 14, v/v), pH (5.0). In the second method; a fluorimetric detection technique was developed, based on precolumn derivatization of VRC using 7chloro-4-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). The fluorescence detector (FLD) was operated at 474 nm for excitation and 539 nm for emission. Isocratic elution was applied with a mobile phase consisting of methanol - distilled water (70: 30, v/v). Separation was achieved using Symmetry® Waters C18 column (150 mm x 4.6 mm, 5µm). Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 0.5– 20.0 µg mL-1 and 0.2 –20.0 µg mL-1 with the first and the second method, respectively. The optimized methods were validated and proved to be specific, simple, and accurate for the quality control of the drug in its pharmaceutical preparation [17].

M. Balaji et al. reported a simple and accurate reverse phase HPLC method has been developed and validated for quantification of VRC and its related impurities in bulk drug and pharmaceutical dosage form. Separation was achieved on c18 column by mobile phase consisted phosphate buffer with pH 3.0 and acetonitrile and flow rate 1.0ml/min. The

detection wavelength was set at 235nm. The method was linear at concentration range from LOQ to 150% of specification level. The limit of quantification and limit of detection values less than 20% of specification level. Method precision and ruggedness the relative standard deviations did not exceed 2%. The accuracy of the method proved, the mean recovery between 85 to 115%. The results demonstrated that the method would have a great value when applied in quality control and stability studies for VRC [18].

Rafal Pietras et al. reported a simple and accurate reverse phase HPLC method has been developed and validated for quantification of VRC in bulk drug and pharmaceutical dosage forms. Herein, an isocratic LC analysis was carried out on a Chromolith Performance RP18e column with methanol-buffer solution pH 3.5 (a buffer solution containing sodium benzoate (0.5 mmol/l) adjusted to pH 3.5 with trifluoroacetic acid (20 mmol/l) (55:45, V/V)) at a flow rate of 1.2 ml/min. The detection wavelength was set at 320 nm. The calibration curve was linear (r = 0.9999) in the studied range of concentration (0.2-10 µg/ml). The selectivity and sensitivity of the elaborated method were satisfactory, and the limits of detection and quantification was less than 20% of the specification level. Moreover, the inter and intra-day precisions was found to be less than 3% (RSD), while the recovery values expressing inter and intra-day accuracy was varied from 99.73 to 101.23. The VRC solution was stable over a period of 3 days on storage under refrigeration. The utility of the developed method was examined by analysing the tablets containing VAR as a result, the method was found to be selective, sensitive, precise and accurate [19].

Liquid-Chromatography-Mass Spectroscopy methods (LC-MS) for VRC

Table 2: LC-MS Analytical methods for VRC

Sr. No	Drug	Matrix	Extraction method	Column	Mobile phase	Mass to charge ratio (m/z)	Ref
1	VRC	Pure form	liquid–liquid extraction	C8 column	acetonitrile:0.001 M ammonium acetate (70:30, v/v)	212.2 for VRC 748.6 for IS	20
2	VRC	Pure form	-	InertSustain C18 column	0.05% trifluoroacetic acid acetonitrile	195.09 for VRC	21

Ayoub Al-Haj et al. reported a method based on liquid chromatography coupled to tandem mass spectrometry was developed for quantitative determination of VRC in human plasma. VRC and the internal standard (25.0 ng/ml of Clarithromycin) were extracted from human plasma by liquid–liquid extraction, using methyl tertiary butyl ether as the organic solvent. The chromatographic separation was achieved using C8 column with isocratic elution using a mixture of acetonitrile:0.001 M ammonium acetate (adjusted to pH 4.0) (70:30%, v/v). The method was validated over the concentration range of 0.1–10.0 ng/mL by investigating specificity, sensitivity, linearity, precision, accuracy, recovery, matrix effect and stability according to United State Food and Drug Administration guideline. The validated bioanalytical method was successfully applied to evaluate bioequivalence of two commercial products of 1 mg VRC single dose [20].

Yuting Lu et al. reported an LC-QTOF-MS method was developed for the separation and characterization of related substances in VRC tartrate. The separation was established on an Inert Sustain C18 column (4.6 mm×150 mm, 5 µm) by liner gradient elution using 0.05% trifluoro acetic acid as mobile phase A and acetonitrile as mobile phase B. The degradation studies were conducted under the ICH prescribed stress conditions.VRC tartrate was found to be unstable to alkaline, oxidative, thermal and photolytic stresses, while relatively stable under acid stress condition. Thirteen related substances were detected all together in VRC tartrate and its stressed samples. Their structures were identified mainly through positive ESI high resolution QTOF mass spectrometric analysis of the parent and product ions' accurate masses and the calculated elemental compositions. Among the 13 related substances, seven were process-related and six were degradation products, and two of them were further verified by chemical synthesis and

NMR spectroscopic determination. Their formation mechanisms were also discussed, and the key steps in the manufacturing processes were also determined to provide VRC tartrate with high purity [21].

Ultra-Performance Liquid-Chromatography (UPLC) Methods for VRC

B. Satheesh et al. reported a new ultra-performance liquid chromatographic (UPLC) method has been developed and validated for quantification of substances related to VRC tartrate, process-related and degradation products, in pharmaceutical formulations. Chromatographic separation of six impurities was performed on a reversed phase column. The method was validated for linearity, limits of detection and quantification, accuracy, precision, and selectivity. The calibration plots obtained for the six impurities were linear over the range 0.005–0.30%. The relative standard deviations of intra and inter-day experiments were less than 1.0%. The detection limits ranged between 0.002 and 0.004%, depending on the impurity. The proposed UPLC method was successfully applied to quantification of VRC impurities in its pharmaceutical formulation [22].

UV-Visible Spectrophotometry Method for VRC

Engin kocak et al. reported VRC is a nicotinic receptor partial agonist used to treat smoking addiction. The objective of this work was to develop and validate UV-Vis spectrophotometric method for the determination of VRC in tablets. In this study, 0.01 M phosphate buffer arranged to pH 7 was used to prepare standard stock solutions from VRC tartrate salt, as well used to dissolve the commercials tablet and synthetic tablet solutions. UV-VIS spectrophotometric determination was performed at 319 nm wavelength having no interference coming from matrix components. The developed method was linear within the range 1-100 µg mL-1. Method validation was performed according to the ICH guideline and the results show that this simple and low cost method is precise, accurate, robust and rugged to be proposed for the routine analysis in quality control laboratories [23].

CONCLUSION:

The present review illustrates various analytical approaches exercised for the estimation of VRC. A several tools had performed including, HPLC, UPLC, Capillary zone electrophoresis, LC-MS methods and UV-Spectrophotometry methods etc. for estimation of VRC in bulk drug. Through this review, we have taken determined efforts to cover maximum literature available on analytical methods of VRC. For analysis of VRC in pharmaceuticals, HPLC with UV detection is appropriate because this method offers accurate and precise results and low cost compared to more superior detection techniques.

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Abbreviations

1. Varenicline (VRC)
2. Gas chromatography (GC)
3. Liquid chromatography (LC)
4. Capillary electrophoresis (CE)
5. Capacitively coupled contactless conductivity (C4D)
6. Diode array detection (DAD)
7. N-formyl VRC (NFV).
8. Capillary zone electrophoresis (CZE),
9. High performance liquid chromatography (HPLC)
10. Liquid chromatograpy-Mass spectroscopy Methods (LC-MS)
11. Ultra-performance liquid chromatography (UPLC**)**
12. Quadrupole time-of-flight (QTOF)
13. Electrospray-ionisation (ESI)
14. Non aqueous capillary electrophoresis (NACE),
15. Capillary gel electrophoresis (CGE)
16. Capillary isoelectric focusing (CIEF)

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